EFFECTS OF INTENSE NOISE ON THE FETAL SHEEP AUDITORY MECHANISM AS ASSESSED BY AUDITORY BRAINSTEM RESPONSE AND COCHLEAR HISTOLOGY

By

LINDA LOUISE PIERSON

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by

Linda Louise Pierson

Dedicated

to

James P. Pierson

a renaissance man

and

my best friend

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The words on the bumper sticker on the car in front of me said, "Expect a miracle." At the time, I thought a miracle might be nice, but now I realize that for my entire life, I have been surrounded by miracles in the form of the people that I have known. My direct thanks and acknowledgment go to the following people and organizations who, knowingly or not, have served in supporting me in the successful completion of this task.

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The auditory brainstem response was recorded from in utero fetal sheep from 111 to 136 days gestational age. Fetuses were prepared for chronic recordings during a sterile surgical procedure. A midline abdominal incision was made in the ewe, through which the fetal head was extracted and instrumented with a bone oscillator and electrodes fixed in the skull. The auditory brainstem response was evoked by clicks and tone bursts of 500, 1000 and 2000 Hertz delivered through the bone oscillator. Pregnant ewes were exposed to an intense broadband noise of 120 decibels sound pressure level for 16 hours while carrying fetuses at gestational ages of either 113 days (early noise-exposed group) or 130 days (late noise-exposed group). A third group of animals was nonexposed.

Auditory brainstem response threshold and latency increases were identified immediately after the noise exposure for the late noise-exposed group. Latency-intensity measurements for three of the four vertex-positive waves identified in the fetal sheep were prolonged ($p < 0.05$) after the noise. Paired t-tests showed that response thresholds for the click stimulus were temporarily elevated in the late noise-exposed group ($p < 0.01$). Although there was no immediate effect on the auditory brainstem response for the early noise-exposed group, when these animals were tested again at later gestational ages, higher thresholds were identified in the noise-exposed group than in the nonexposed animals (one-way analysis of variance, $p < 0.05$).

Serial celloidin sections of the cochleae were prepared from randomly-selected noise-exposed and nonexposed fetuses. Cochlear reconstructions and cytocochleograms were completed. The sheep cochlea has an unusual shape, with a long basal turn and a curved modiolar axis. Light microscopy analysis of the organ of Corti showed unavoidable preparation artifact because of the unusual cochlear shape. With the exception of the preparation damage, the cochleae of the nonexposed and the noise-exposed groups had a similar appearance.

Results showed that exogenous sounds can penetrate the uterus and that these sounds result in alterations of the fetal auditory brainstem response. Light microscopy did not show sensory cell differences between noise-exposed and nonexposed animals.

CHAPTER I
INTRODUCTION

Noise is everywhere in contemporary society, permeating the environment in which people work and relax. Noise is known to elevate stress levels, reduce work efficiency and, if sufficiently intense, reduce hearing sensitivity. The relation between occupational exposures to intense sound and decreases in hearing acuity was first reported in the early 18th century (Ramazzini, 1713/1964) and, since then, has been widely studied. A 1991 U.S. Public Health Service objectives document reported that noise exposure is responsible for the hearing losses of approximately 10 million of the estimated 21 million Americans with this disorder. In 1990, it was reported that more than 20 million Americans are exposed on a regular basis to hazardous noise levels that could result in hearing loss (NIH, 1990).

Significant numbers of these noise-exposed Americans are women of childbearing age (Moss and Carver, 1992). Although hearing loss has been documented in unprotected individuals exposed to hazardous levels of noise, the effects of these exposures on the fetuses of pregnant women are unknown.

Noise-Induced Hearing Loss

Noise-induced hearing loss (NIHL) is described as sensorineural in origin, affects the high frequencies, occurs insidiously, and worsens with continued exposure. The severity and the region of cochlear damage largely depends on the duration, level, spectral, and temporal characteristics of the noise exposure. Noise "level" refers to the actual sound pressure level (SPL) of the stimulus, whereas, noise "exposure" refers to the combination of noise "level" and duration of the stimulus (Lipscomb, 1988).

Assessment of NIHL Damage

Obtaining measurements of damage to the hearing mechanism following hazardous-noise exposure is not always straightforward. The relation between cochlear damage and measurements of hearing is neither direct nor one-to-one. Pure-tone behavioral thresholds are uncertain predictors of type and degree of inner ear pathology. In animal experimentation, behavioral measurements have the added disadvantage of the time needed to train animals to correctly respond to signals. Two popular methods for obtaining information about hearing mechanism status in animal preparations following noise exposure are the auditory brainstem response (ABR) and histological analysis.

The ABR. Animal studies of NIHL frequently use the ABR to obtain a measure of change in hearing sensitivity.

Most studies measure the ABR threshold, but data from latency-intensity functions or latency-amplitude functions are also used to provide information about cochlear damage. Popelar et al. (1987) used auditory evoked potential recordings from the inferior colliculus and found threshold shifts as well as changes in amplitude-intensity functions.

Coats and Martin (1977) were among the first to suggest, in humans, an effect on the ABR with cochlear hearing loss. They reported that high frequency hearing loss affected the ABR waveform by prolonging Wave I latency more than Wave V latency. Additionally, the latency-intensity function for adults with cochlear hearing loss is steeper and usually shorter than for normal hearing adults (Galambos and Hecox, 1978; Yamada et al., 1979; Picton et al., 1981). The main features of cochlear-damaged ABR are: (1) slope of the latency-intensity that increases immediately above threshold; (2) normal latency for mild and moderate losses; (3) higher correlation between ABR threshold and behaviorally measured thresholds as the loss increases.

Additional studies have dealt with the relative importance of audiogram configuration and behavioral thresholds to pure tones (Jerger and Mauldin, 1978; Yamada et al., 1979; Keith and Greville, 1987). The amount of hearing loss as well as the configuration of the audiogram affects the appearance of the ABR.

Cochlear changes

Boettcher et al. (1987) summarized possible structural changes in the cochlea following exposure to hazardous noise. Noise can damage a variety of structures in the cochlea, including the outer and inner hair cells, supporting cells, blood vessels, secretory cells, and neurons. At lower levels of hazardous noise exposure, hair cells and afferent dendrites may be damaged by high rates of metabolism (Saunders et al., 1986; Pujol et al., 1986). As the level of exposure increases, metabolism may be a lesser factor and cochlear mechanics may play a greater role in producing cochlear damage. Intense noise causes tight cell junctions to rupture, allowing perilymph and endolymph to mix, thereby destroying the electrical and chemical balance in the hair cells and causing cell death (Bohne and Rabbit, 1983). At still higher levels of continuous noise (greater than 120 decibels on the A-scale, dBA) or impulse noise, the cochlea can be damaged primarily by mechanical processes, e.g. by ripping Reissner's membrane or by tearing the basilar membrane (Voldrick and Ulehova, 1982).

Borg and Engstrom (1989) found structural differences in the cochlea when they compared short-term, high-level exposure (115 SPL for 30 minutes) and long-term, low-level exposure (85 dB SPL for 512 hours). With the high-level, short-term noise, they found wide-spread damage to the inner hair cells (IHC), fusion and inclination of the tallest stereocilia and the loss of a few outer hair cells (OHC), predominately in the 2 to 8 kilohertz (kHz) region. Conversely, exposure to the low-level, long-term noise showed no damage to the IHCs, but pronounced loss/damage of the OHCs as well as a very different audiometric configuration with damage in 12 to 20 kHz region. They speculated that differing damage could be related to the degree of contact between of Hensen's stripe the IHC stereocilia. During exposure to high noise levels, the increased contact between the tectorial membrane and IHC stereocilia could lead to fatigue and ciliary fracture. Henson's stripe exerts direct force on the stereocilia and fatigue of the stereocilia caused by mechanical bending may increase rapidly as a function of vibration amplitude. This supports the idea of a critical level where damage to the IHCs can occur. The findings of OHC lesions at low-level are compatible with the hypotheses of metabolic fatigue for low and moderate-sound levels.

In an attempt to describe and quantify the type of damage occurring in the cochlea, Rydmarker et al. (1989) defined 10 different hair cell abnormalities: (1) The absence of cuticular plate and presence of phalangeal scar. This has been the most widely reported morphological damage reported from noise exposure (Soudijn, 1976; Engstrom and Engstrom 1978); (2) Bent stereocilia; (3) More than two stereocilia absent; (4) Stereocilia that had lost contact with adjacent stereocilia but were not bent (floppy appearance); (5) Fused stereocilia, but still standing up; (6) Giant stereocilia (without fusion); (7) Stereocilia fusion with elongation; (8) Shorter than average length of the area on the basilar membrane where located; (9) Dissolved into the cuticular plate (melted); and (10) Cuticular plate protruded from the plane of the reticular lamina, with or without stereocilia.

Pickles et al. (1987) studied the effects of intense sound (117 dB SPL at 10 kHz for 2 hours) on stereocilia linkage in guinea pigs. When the stereocilia and their lateral links were not disarrayed by sound, the tip links remained intact, even if the hair cells were close to more severely damaged hair cells. If the spacing between stereocilia was increased, tip links were more likely to have been lost. Pickles et al. (1987) hypothesized that the tip links were initially less vulnerable than lateral links or the internal core of the stereocilia. However, when lateral links or the core were damaged, tip links fractured. The IHC pattern was different than for the OHC. In OHC, different rows of stereocilia and their tip links seemed equally vulnerable to damage by sound. In the IHC, the tallest stereocilia were easily detached from the next row, with complete fracture of their lateral and tip links to the next row, while the tip links between the shorter stereocilia remained intact. According to Pickles et al., this could happen to IHC stereocilia, if the stimulus were preferentially coupled to the tallest stereocilia. Once the links joining them to the next row were broken by an intense stimulus, the stimulus could not be coupled mechanically to the shorter stereocilia, and they and their links would be isolated from further damage. If these tip links survived, the hair cells could continue to transduce the signal. Since it seems that the stereocilia on the IHC are moved by viscous drag of the surrounding corti lymph, there would still be some mechanical coupling of the stimulus directly to the shorter stereocilia even if the taller stereocilia were no longer functioning normally. After acoustic trauma to the IHCs, auditory nerve fibers, and so presumably the IHC to which they are connected, could continue to respond to a reduced level of sound. Canlon et al. (1987) also studied the effects of intense sound on stereocilia. They discovered that, after noise exposures, the IHC stereocilia became less stiff and it took less force to move the stereocilia. No structural alteration of the stereocilia bundles was obvious, but Canlon et al. speculated that structural differences between the IHC and OHC, perhaps in stereocilia core, tip links or the cuticular region, could be responsible. Another possible cause for the differential reaction in stiffness could be the interaction between the rootlet and the cuticular plate, thus affecting the contractile mechanism.

Alterations in the tectorial membrane were reported by Canlon (1988) after guinea pigs were exposed to a 1 kHz tone at 105 dB SPL for 72 hours. She found alterations of the fiber structure of the middle zone of the tectorial membrane, discontinuities in Hensen's stripe, reduced thickness of the tectorial membrane, localized reduction of IHC stereocilia stiffness, swollen afferent dendrites beneath the IHCs throughout the second, third, and fourth cochlear turns, swollen supporting cells surrounding the IHCs, OHC damage such as an increased number of Hensen bodies, swollen cell bodies, and swollen cuticular plates, and a loss of sensitivity with the maximal threshold shift seen between 2 and 8 kHz. She speculated that the changes in the tectorial membrane could have been caused by dehydration, depolymerization of fibers as result of mixing inner ear fluids or mechanical stress of the impact of the tectorial membrane against the stereocilia, causing changes to fibers and Hensen's stripe.

The relation between ABR and histological analysis. In 1987, Borg reported that immediately after exposing rats to 105 dB SPL wide-band noise for 12 hours per day for 1 month, there was little or no significant loss of hair cells corresponding to hearing loss. A 3-month exposure caused widespread moderate loss of outer and inner hair cells. After the 7-month exposure, there was good agreement between hair cell loss and amount of hearing loss, measured both behaviorally and by the ABR. After 15 months of noise exposure, hearing loss had progressed, but the loss of OHCs had progressed even more and IHC loss had stayed about the same.

Hamernik et al. (1989) attributed different behavioral results to nonhomogeneous pathologies and to the fact that the psychophysical threshold reflects the totality of changes that have taken place at the periphery as well as centrally, and not simply sensory cell loss. They identified behavioral results with 20-40 dB hearing loss with no sensory cell loss and essentially normal thresholds in the presence of OHC lesions. Rydmarker et al. (1987) wrote that minor damage to hair cells can be observed without changes in hearing sensitivity. An impact noise for 3 hours did not cause significant auditory threshold shift as measured by electrocochleography from guinea pigs. However, there was identifiable hair cell loss. When there were increased threshold shifts to a 12-hour impact exposure, there was an increase in minor hair cell damage, but only a small increase in the number of missing hair cells. In another study, Price et al. (1989) stated that following noise exposure and a recovery period, lesions could be seen histologically even when behaviorally measured thresholds had returned to pre-exposure level. They hypothesized damage at the cellular level even though some measures of sensitivity did not show it. If the assumption of a critical level for damage from impulse noise exists, then in a condition near the critical level for an average ear, less susceptible ears would be below this individual critical level and would experience no losses. Conversely, other individuals who are well above the critical level would experience serious losses.

Price et al. (1989) cautioned that hair cells may play a major role in determining the stiffness of the organ of Corti, which determines the frequency at which a particular place is resonant. Therefore, assigning a particular place on a cochleogram to a tonal frequency in a damaged ear may not have the same meaning as assigning frequency in an undamaged ear.

Borg and Engstrom (1989) questioned the underlying assumptions of using audiometric values to interpret cochlear site of damage. Traditionally, it was believed that hearing loss grows at the frequency in question, rather than damage being shifted in frequency. Findings that show different frequency of hearing loss for different levels of exposure support the argument against the traditional view of a one-to-one cochlear place/frequency relation. Thus, the use of averages over the entire frequency range or several selected frequencies may be preferable than selecting one or more points to express cochlear damage.

In Canlon's 1988 study, inner hair cell stereocilia, once affected to a certain point, did not show further decrease in stiffness with increasing exposure duration. In contrast, ABR thresholds showed dependent change in sensitivity with increasing exposure duration. Secondly, in her study, IHC stereocilia stiffness returned to pre-exposure thresholds after a recovery period, while ABR thresholds were still decreased by 25 dB. She concluded that it is an oversimplification to assume that only the IHC stereocilia are responsible for decreases in auditory sensitivity. Even though the IHCs have most of the afferent connections, the OHCs contribute to the improved auditory sensitivity. Lonsbury et al. (1987) found that their low level of OHC loss in all animals correlated poorly with more extensive frequency pattern of behavioral threshold shifts, also suggesting that extensive threshold shifts cannot be accounted for by stereocilia alone.

On the other hand, Davis et al. (1989) found a systematic relation between the degree of permanent threshold shift, abnormal OHC populations and changes in the quality of tuning. They reported detectable changes in the tuning curve (Q 10 dB, high-frequency slope and low-frequency slope) when permanent threshold shift exceeds 10 dB, and concluded that changes in tuning are related primarily to systematic loss of OHCs.

A lack of correlation between hair cell loss and electrophysical results could be explained by the nature of IHC loss. Damage affecting only a small percentage of IHCs will shift some of the tuning curves (Engstrom and Borg, 1983). The firing rate in the nerve fibers from adjacent normal IHCs could increase sufficiently to compensate for the shifted tuning curves and leave the ABR and behavioral thresholds largely unaffected. If the damage affects a greater number of IHCs and the corresponding tuning curves in a particular region, the auditory thresholds would be shifted. In this case, there would be too few intact nerve fibers in the immediate vicinity to compensate for those with the shifted thresholds.

Fetal Hearing

Development

Embryologically, the ear matures from an ectodermal thickening, called the auditory placode. The placode develops into a vesicular shaped pit, termed the otocyst. In humans, at about 4 to 5 weeks gestational age (GA), the otocyst divides into two lobes, the labyrinth and the cochlea. At 6 months, both the organ of Corti and the tunnel of Corti are present in all turns of the cochlea and by about the 24th week, the cochlea and peripheral sensory end organs have reached their normal development (Gagnon, 1989). According to Querleu et al. (1989), the human cochlea obtains full morphological development by the 10th week and is adult size by the 20th week. It can be inferred by parallel chronology from other species that the human organ of Corti is functional by 18 to 20 weeks. By this time, the auditory neural pathways are functional, although the myelination process that starts during the 20th week of fetal life is not completed at birth.

It appears that normal growth and maturation of the brain depends on an intact auditory system. This was demonstrated by the impairment of glucose utilization in the auditory as well as nonauditory portions of the brain after cochlear ablation in fetal sheep (Abrams et al., 1987). Harris et al. (1990) found that in the gerbil, cessation of growth of the cochlea occurs before acoustic stimulation first evokes cochlear responses and that cessation of basilar membrane growth immediately precedes the onset of function.

Walsh and McGee (1990) suggested three stages of development in cats. During the first stage, thresholds are very high and well outside of the range of naturally occurring acoustic events. Response sensitivity does not significantly improve during this period, and the response frequency range is restricted to low- and mid-frequencies. They believe that the second stage is characterized by rapid improvements in threshold and acquisition of adult-like organization. Changes during this stage would include epithelial reorganization of what appear to be elements that impart mass and stiffness to the basilar membrane, the formation of hydraulic fluid spaces and adult-like tectorial membrane, and organ of Corti innervation patterns. During the third stage, the remaining components mature and myelination is completed.

Rubel (1984) wrote that "no single event triggers the onset of cochlear function" (pg. 216). Before the onset of function, there are many simultaneous and synchronous events contributing to the maturation of mechanical and neural properties. These events include a thinning of the basilar membrane, formation of the inner spiral sulcus, maturation of the pillar cells, freeing of the inferior margin of the tectorial membrane, development of tissue spaces in the organ of Corti, differentiation of the hair cells, establishment of mature stereocilia structure, and the maturation of the synapses. This synchrony of development was observed with mice in 1978 (Rubel). The majority of cell types underwent terminal cell mitoses on day 14 GA. These cell types included spiral ganglion cells, IHCs, OHCs, inner pillar cells, outer pillar cells, Deiter′s cells, Hensen′s cells, Claudius′ cells, inner supporting cells, and external sulcus cells. Rubel suggested either a common origin or some active process regulating the synchronization of proliferation.

These events do not occur at the same time throughout the length of the cochlea. Two developmental gradients exist. The first is a longitudinal, basal to apical development. Additionally, a radial development from modiolus to stria vascularis occurs. Longitudinally, in humans, differentiation occurs first in the mid-basal region and spreads in both directions, with the apex maturing last. In radial development, synapses form early on IHCs and change little thereafter. The OHCs mature later than the IHCs and are initially surrounded by afferent terminals which are gradually replaced by numerous efferents. Then, the large calyciform efferent terminals form, which are typical of the mature cochlea.

Consequently, there seems to be some sort of "autoregulation," that allows simultaneous growth and destruction of cell types that leads to a cochlea that is electrically, chemically, and mechanically capable of rudimentary function.

Rubel and Ryals (1983) found in chicks that during the late stages of maturation, the position of hair cell damage on the basilar membrane shifts apically with an exposure to an intense 1500 Hz pure tone. The exposure of older animals caused the region of hair cell loss to be shifted toward the apex. These results suggest that the region of the basilar membrane that is maximally activated by a relatively low frequency tone has a basal location initially and then, with increasing age, shifts toward the apex. This shift could be caused in part by the mechanical changes secondary to increases in the stiffness and mass of the basilar membrane.

The development of the active process may be the main cause of the frequency/place coding shift.

Norton et al. (1991) reported an orderly progression in the appearance of otoacoustic emissions during gerbil development. They concluded that "initially cochlear mechanics are passive and that active elements associated with normal OHC function mature first in the basal turn and last near the apex" (pg. 73). Walsh and McGee (1987, 1990) argued that additional support for OHC maturation after the system has begun to function is provided by the auditory nerve tuning curves recorded from neonatal kittens. These recordings exhibit high thresholds, saturation levels in excess of 125 dB SPL, small dynamic ranges and low maximum discharge rates. These characteristics are strikingly similar to the input-output curves of noise-exposed ears in adult animals.

Studying guinea pigs, Pujol et al. (1991) found that the appearance and development of the OHC motilities *in vitro* follows a base-to-apex gradient. Prior to onset of motility, OHCs acquire an adult-like distribution of microfilaments and intermediate filaments. Also, the onset of motility coincides at gross morphological level with an increase in the length of the cell and its regular cylindrical shape. They felt, however, that the most prominent ultrastructural OHC changes are at the level of the subsurface cisternae complex. The cisternal complex, at the onset of motility, corresponds to a regular alignment of one layer of cistern along the lateral plasma membrane.

The intrauterine sound environment

Typically, most noise exposures occur in an air medium. The fetus is an exception in that the sound energy must pass from the air medium to the fluid medium of the amnion. As it changes media, sound energy is reduced because of the impedance difference at the air-tissue interface. The acoustic impedance of water is much higher than air. For a given pressure disturbance, the particle velocity is much less, by a factor of approximately 3600. Therefore, equal pressures in air and fluid differ in sound energy by approximately 36 dB (Gerhardt, 1990). Thus, one would assume that the sound pressure required to produce a physiologic response from the fetus would be approximately 36 dB greater than the sound pressure in air necessary to produce the same response from the newborn (Gerhardt et al., 1992). Factors that determine how much *ex utero* sound reaches the inner ear of the fetus include the sound pressure attenuation through maternal tissue and fluid and the transformation of these pressures into basilar membrane displacement.

Digestive and respiratory sounds dominate the internal recordings obtained in the amnion of a pregnant ewe (Gerhardt et al., 1990). In their study, the intrauterine environment was monitored with a hydrophone. Sounds of mastication, swallowing, and physical movement were easily detected above the noise floor and conversations between experimenters three feet from the ewe were recognizable. Speech was muffled and intelligibility was poor. However, pitch, intonation and rhythm were clear, suggesting that the maternal voice forms a prominent part of a rich intrauterine sound environment.

The observations above as well as hydrophone measurements suggest that intrauterine sound environment functions as a low-pass filter. Measuring the sound environment of pregnant sheep with a hydrophone, Gerhardt (1990) and Gerhardt, Abrams, and Oliver (1990) reported that the sound environment is dominated by frequencies below 500 Hz. For frequencies below 250 Hz, the reduction in sound pressure through maternal tissues and fluids was less than 5 dB. Above 250 Hz, attenuation increased at a rate of 6 dB per octave up to approximately 4000 Hz, where the average attenuation was 20 to 25 dB. Therefore, sound transmission to the fetal head through the intrauterine tissue is more efficient for low frequency sounds than the higher frequency sounds.

The amount of basilar membrane displacement with fetal sound stimulation is less well understood. Normal adult auditory function requires an air-filled middle ear cavity, an intact tympanic membrane, and functional hair cells and neural mechanisms. Basilar membrane displacement, caused by the hydraulic motion of the cochlear fluids, must occur for IHCs and OHCs to be stimulated. In the normal adult system, stapedial movement in and out of the oval window sets the fluids in motion. The fetal middle ear and external ear canal are filled with amniotic fluid, which decreases the mechanical advantage of the middle ear. In addition, sound pressures may be present with the same phase at the two windows. The lack of a phase difference, in addition to the lack of a middle ear amplifier may substantially decrease basilar membrane displacement, and therefore cause a decrease in hearing sensitivity.

On the other hand, sound may be efficiently transmitted from the outer ear through the middle ear to the inner ear. Querleu et al. (1989) wrote that

> The middle ear is not necessary for fetal audition. Because the outer and middle ears are filled with amniotic fluid and because liquids, tissues and bones have close conducting properties, the acoustic energy inside the uterine cavity can reach the cochlear receptors with negligible energy loss in utero. Thus, there is no need for an amplifying system. (pg. 410)

Because all cavities are filled with fluid, there is less impedance mismatch between the outer ear and the inner ear, and less need for the impedance matching function of the middle ear. However, the phase of the sound pressure at the oval and round windows is still important. If equal pressures to both windows are in phase, an inertial lag in stapedial movement will not occur. Without a lag in the vibration of the stapes footplate with respect to the skull, basilar membrane displacement will not occur or will be greatly reduced. Without knowing the effect of the intrauterine environment on stapedial movement, the amount of fetal basilar membrane displacement caused by a known sound pressure at the fetal head cannot yet be determined.

Assessment of Fetal Auditory Function

Assessment of fetal auditory function can be accomplished by histological and/or electrophysiological methods. Recent studies using electrophysiological methods have allowed *in utero* measurements of the ABR in pregnant ewes. Sheep have been used in these types of studies not only because pregnant sheep have similar dimensions to pregnant women, but also because of the similarity in adult sheep and human minimum audible field measurements (Wollack, 1963; Heffner and Heffner, 1985). Woods et al. (1983) obtained fetal sheep ABRs using a modified hearing aid amplifier to transduce the stimulus. In later studies, Woods and Plessinger (1985) and Woods et al. (1987) reported the observation and latencies of five vertex positive waves for fetuses of GAs of 117 to 126 days and lambs of GAs up to 144 days. No repeatable ABR response could be identified before a GA of 117 days. Cook et al. (1987) used a similar preparation with a hearing aid transducer and also observed consistent responses no earlier than 117 to 118 days. In their study, Waves I, II, and III were observable at 118 days when the ABR was first elicited and Waves IV and V were observed several days later.

The modified hearing aid transducer may not have provided the best stimulus for obtaining the ABR. Wolfson (1990) obtained bone-conducted ABRs from sheep fetuses *ex utero* as early as 106 days GA. In this preparation, the fetuses were delivered, and maintained on a life-support system while the ABR was recorded. Wolfson (1990) reported age-related changes in the ABR, suggestive of substantial brain-stem maturation between 106 and 122 days gestation.

Gerhardt et al. (1992) recorded in utero cochlear microphonics (CM) from fetal sheep at 109 days. Presumably, the CM precedes the first appearance of the auditory brainstem response in sheep as it does in other precocious animals. The CM recordings from animals younger than 109 days GA were not attempted for this study.

Fetal noise-induced hearing loss

Evidence concerning the existence of fetal noise-induced hearing loss is equivocal. Dunn et al. (1981) exposed pregnant sheep to 130 dB SPL broadband noise for 4 hours a day, 5 days a week, for 5 months. After delivery, measurements of ABR thresholds from the 30- to 40-day old lambs were not significantly different from ABR thresholds of a control group. Although morphological changes were found to be twice as frequent in the noise-exposed group, preparation artifacts could not be excluded as a cause of observed cochlear damage.

In contrast to the Dunn study, a 1991 fetal sheep study (Pierson et al., 1991) measured the ABR *in utero* before and after a 16-hour, broad-band noise exposure. They identified significant changes in ABR latencies elicited by a broadband click stimulus. Whenever there was a significant difference between pre- and postexposure measurements, the postexposure measurements had a longer latency.

Cook et al. (1981) exposed a group of pregnant guinea pigs in the last trimester of pregnancy to 115 dBA of tape-recorded loom noise and after birth, compared ABR latencies to a nonexposed group. They reported consistent Wave IV latency differences that they attributed to "direct overstimulation of the developing auditory system" (pg. 101) or to "abnormal pathway development following noise exposure during critical maturation periods" (pg. 101).

Additional support of the possibility of hearing changes subsequent to noise exposure was provided by Lalande et al. (1986). They found an increased risk of hearing loss in children whose mothers were occupationally exposed to hazardous noise. In their study, there was a significant increase in the children's risk of hearing loss at a frequency of 4000 Hertz (Hz) when the exposure involved a strong component of low-frequency noise.

Some researchers have expressed concern about antepartum fetal stimulation with an electronic artificial larynx (EAL) (Gerhardt, 1989; Gerhardt et al., 1988). Noise levels from the EAL in the intrauterine environment averaged between 101 and 134 dB depending on the distance from the hydrophone. These levels are well above occupational levels considered potentially hazardous to unprotected hearing, depending on duration (Occupational Safety and Health Administration, 1981).

The critical period

Cook et al. (1981) suggested that a critical period of auditory development appears to coincide with or closely follows the finalization of auditory maturation processes. Some researchers have found increased susceptibility of NIHL at particular periods of auditory development. These investigations involve the study of a variety of animal species with undeveloped hearing mechanisms at birth. Consequently, generalizing results to humans is difficult. However, there are similarities in NIHL susceptibility among these animal species.

Bock and Saunders (1977) identified, in hamsters, increased CM thresholds after a 125 dB SPL octave-band noise exposure at a critical age. The greatest increase in threshold after noise exposure occurred during a period of CM threshold improvement in the nonexposed control animals. Price (1972, 1976), using cats, also found that young animals were more susceptible than adults to changes in the CM after a 104 to 124 dB SPL, 5 kHz tone for 50 minutes. Saunders and Hirsh (1976) determined a similar critical period for an increase in CM threshold in mice. In the rat, Lenoir et al. (1979) found the maximum effect of noise on the action potential (AP) threshold measurement occurred at 22 days. The time course of the increased susceptibility appears to vary by species, but appears to follow a pattern of maximum susceptibility some time after onset of auditory function, instead of concurrently with the onset of function. Saunders and Bock (1978) could not speculate on the cause of the time course of the critical period since at the time of increased NIHL, the cochleae and physiological responses appear developed.

In a histological study, Falk et al. (1974), identified more IHC and OHC in young guinea pigs than in adult guinea pigs 1 month after noise exposure. Within the young animals, there was a trend towards greater amount of cellular damage in the group exposed on the second day than in the group exposed on the eighth day.

It appears that, at least in species with immature hearing mechanisms at birth, there is a period of increased susceptibility to noise exposure. This period of increased susceptibility with species that develop hearing *in utero* is not well understood.

The objective of this study is to contribute to the understanding of *in utero* hearing development by investigating the *in utero* development of hearing in fetal sheep, determining the effect of a noise exposure during what may be a critical period of development, and observing hair cell status in both the normal and noise-exposed sheep fetus.

CHAPTER 2
OBJECTIVES AND METHODS

The overall aim of this project was to evaluate the hazards of over-exposure to noise on the *in utero* development of the auditory system of fetal sheep. Changes produced by noise exposure at two different GAs were evaluated through the use of electrophysiologic and histologic methods. Auditory function was assessed from ABR recordings to click and tone burst stimuli. Cochlear changes were assessed by a histologic method of reconstructing cytocochleograms from serial celloidin sections. To meet the above aim, two experiments, one longitudinal and one cross-sectional, were completed.

The purpose of the first experiment was to assess the effect of noise-exposure at a particular GA on auditory system development. In this experiment, the functional and structural development of the auditory system of noise-exposed fetuses were compared to the functional and structural development of the auditory system in non-exposed, age-matched fetuses from 111 to 136 days GA. The ABRs at various GAs were recorded and compared between groups. In addition, animals were sacrificed and the cochleae removed for histopathologic descriptions of exposed and nonexposed fetuses.

The purpose of the second experiment was to determine if delivery of an intense noise to an early GA fetus versus a later GA fetus creates differences in ABR responses and cochlear structure. In this experiment, fetal ABRs were recorded and compared before and after noise exposure at two different GAs. Five days after noise exposure, the cochleae from representatives of these two groups of fetuses were removed, prepared and sensory cells were described.

Subjects

Thirty-four, time-mated pregnant ewes provided fetuses as subjects for these two experiments. The ewes were housed in individual stalls (4′ by 8′) for 4 to 5 days before surgery to allow them to acclimate to the environment. Following surgery and each treatment condition, ewes were returned to these stalls. At the conclusion of the experiment, ewes were euthanized with a concentrated barbiturate solution administered through previously implanted intravenous catheters. Temporal bones from selected subjects were removed rapidly and prepared for histologic analysis.

Gestational ages at the time of noise exposure were 113 and 130 days (+/- 2 days). Subjects numbered 005, 008, 010, 012, 013, and 014 of a previous experiment comprised the "late-exposed" group (N = 6). These animals were surgically instrumented at 127 days, and provided ABRs before and after exposure to noise as well as after a 5 day recovery period. The cochleae of two subjects were harvested at 136 days GA.

The "early-exposed" group consisted of 13 ewes surgically prepared at 110 days. The surviving ewes were functionally tested at ages of 111, 113 (preexposure), exposed to noise at 113 and also functionally tested at 114 (postexposure) 116, 130, 131, and 136 dGA. For the cross-sectional study, two of the animals were sacrificed at 119 dGA, cochleae removed and prepared for histology. Of the remaining 11 animals, six subjects were evaluated through 130 days GA, five of these six animals were evaluated through 136 days and two of these animals provided cochleae for histology.

The "nonexposed group" consisted of nine animals surgically prepared at 110 days GA. The ABR testing was completed on surviving animals according to the same schedule as the early-exposed group. Six animals were evaluated through 130 dGA and five animals completed the protocol through 136 dGA. The cochleae from two animals sacrificed at 136 days GA were prepared for histology.

Animals in the "surgery only" group (N = 2) were instrumented, were not tested and cochleae were removed and prepared for histology at 136 days GA. In the "histology only" group (N = 4), two subjects were sacrificed at 119 days GA and two subjects at 136 days GA. Cochleae were removed and prepared for histology.

Procedures

Surgery

To record ABR from the *in utero* sheep fetus, sterile surgery was required to apply the necessary chronic instrumentation. The ewes were brought into the surgery room and anesthetized with halothane. All equipment and instruments had been gas sterilized. The fetal head was exteriorized through a cesarean incision. The fetal skull was exposed by retracting overlying tissue, and screw-electrodes were secured midline nostral to the frontal bones, in the vertex and in both mastoids on or near the head of the styloid process. To provide insulation and additional stability, the electrodes were covered with methyl methacrylate. The bone oscillator was secured central to the fetal skull on the parietal and occipital bones as far caudal as possible. Two rows of three screws each were fixed in the skull and the oscillator was secured between them with thread.

The incision was closed over the electrodes and around the bone oscillator. Also, a catheter was placed in the femoral artery of the ewe. The fetus was returned to the uterus and the uterus and abdomen of the ewe were closed. All wires were passed under the maternal skin and out through an incision in the maternal flank. Electrode leads, wires and the catheter were stored in a pouch sutured to the maternal flank. To protect the pouch, ewes wore an elastic piece of material around their bodies over the pouch.

The ABR

Equipment

Electrical signals from the fetus in response to acoustic stimuli were recorded by the Tracor Northern (Model TN3000) computer averaging system. Signals were differentially recorded, amplified (100,000 fold), filtered (0.1-3.0 kHz), averaged and stored on disk for later analysis.

Six bone oscillators (Radioear B70A), closely matched for output and labeled A, B, C, 81, 743 and 23179, were used to provide the acoustic stimulus to the fetal heads. Each oscillator was sealed with acrylic around the seam and wire connection to preclude fluid damage. The outputs of the bone oscillators were calibrated with an accelerometer fixed directly to the vibrating surface. Accelerometer output was directed to a spectrum analyzer (Bruel and Kjaer, B&K, model 2123).

The amplitude and spectral characteristics of the bone oscillators was compared for each bone oscillator for each of the four stimuli (broadband click, 4 kHz, 2kHz, and .5 kHz tone bursts) used to evoke the ABR. The time waveforms for these stimuli and their spectra for bone oscillator labeled 743 shown on a one-twelfth octave band scale appear in Figure 2-1. Stimulus levels for the fetus can only be estimated because of the direct placement of the oscillator on the skull as well as the presence of a fluid-filled environment. Stimulus level (dB nHL) was referenced to young normal human (N = 5) behavioral thresholds with a bone oscillator placed on the mastoid.

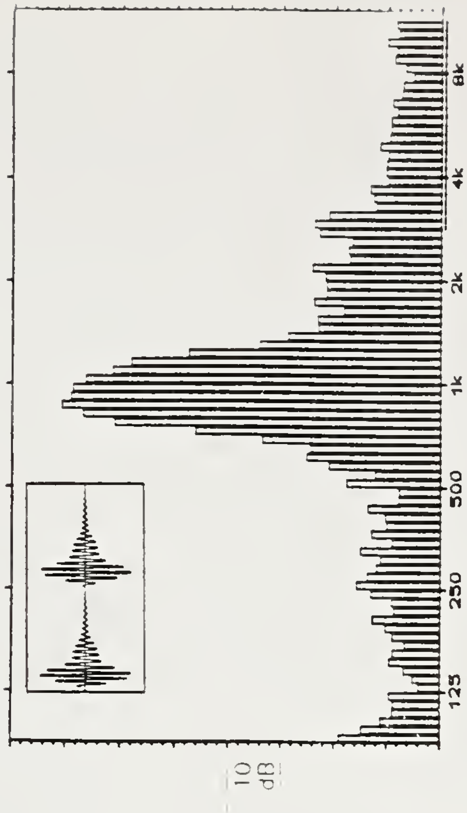
Recording the ABR

With the exception of one animal that was tested 6 hours after surgery, all testing from fetuses occurred at least 1 day after surgery. On all days of ABR testing, the ewe was placed in a cart and positioned in a sound-treated room. The fetal electrodes were connected to the Tracor Northern ABR averaging unit. The two electrode leads positioned in the mastoids were linked together and connected to the inverting input of the amplifier. The vertex electrode was connected to the non-inverting lead of the amplifier and the snout electrode was connected to the ground input of the amplifier. The ABR was sampled for 2000 trials per average over an 18 millisecond (ms) window for tone bursts and a 10 ms window for clicks. Broadband clicks with a 0.1 ms duration and tone bursts of 2 kHz and 1 kHz with a two cycle rise/fall time and one cycle duration were presented at a click rate of 21 Hz. In addition, a 0.5

Figure 2-1. Time waveforms and spectra for ABR stimuli recorded with an accelerometer:

A. 0.5 kHz tone burst
B. 1 kHz tone burst
C. 2 kHz tone burst
D. 100 microsecond click

Time waveforms are shown over 40 ms. Spectra are shown in one-twelfth octave bands.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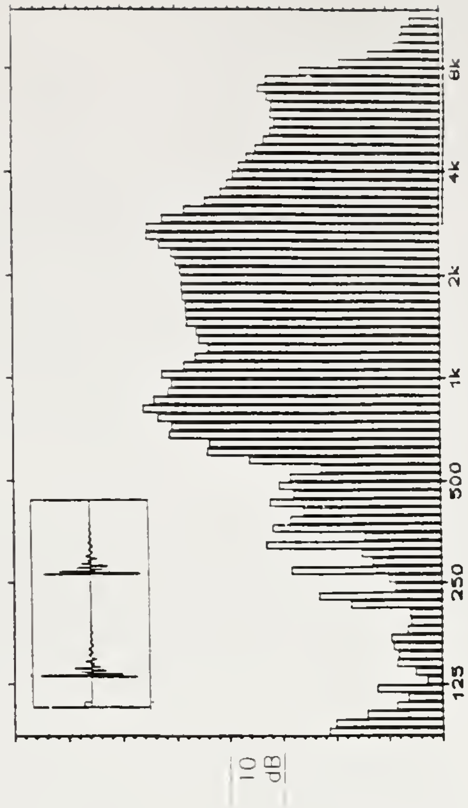
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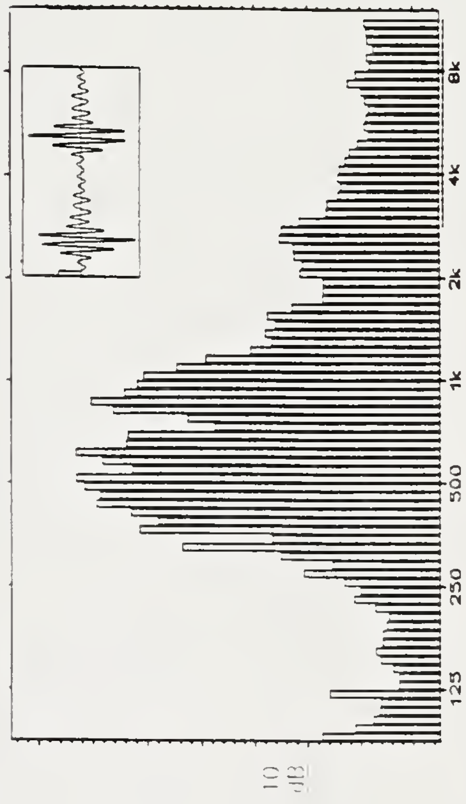
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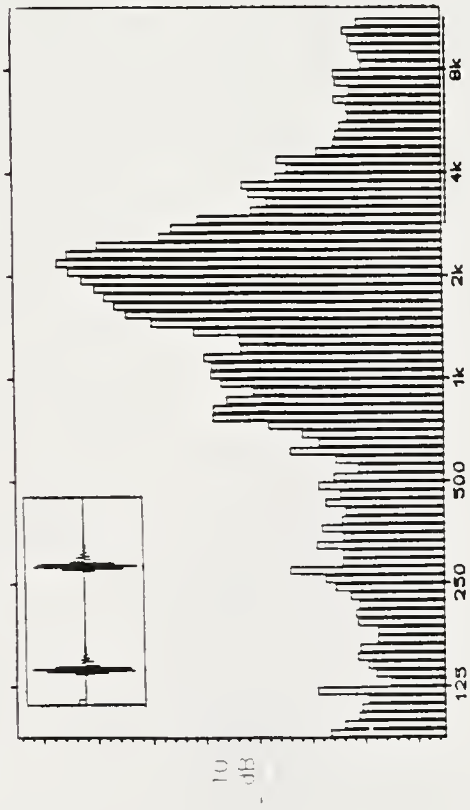
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kHz tone burst with a 2 cycle rise/fall time, but a 0.01 ms duration was presented.

Responses were recorded starting at 80 dB on the Tracor unit and decreased in 20 dB steps until the response was no longer identified. Then the level was increased by 5 and 10 dB steps until an ABR threshold was reached. Replications of all responses were completed to support absolute latency and threshold identification.

The Noise Exposure

Equipment

The noise exposure was presented in an 10′ by 11′ sound-treated booth (Industrial Acoustics Company, model GDC-1L). A 120 dB SPL, broad-band noise was delivered to the sides of the ewe through four loud speakers. Speakers were placed near both flanks as well as above and behind the back of the ewe. A B & K sound level meter (model 2209) with a B & K one/half inch microphone was used to check the noise levels around the ewe before the exposure begins. At the maternal flank, noise levels within 1 dB of 120 dB SPL were accepted.

Completing the exposure

After the appropriate preexposure ABR, the ewe remained in the cart in the sound-treated room, and was exposed to

120 dB SPL broadband noise sound-field for 16 hours. Maternal hearing was protected by foam hearing protectors placed in the external ear canal and secured with tape around the pinna.

Histological Methods

Obtaining the cochleae

Cochleae from fetuses were obtained at the designated GA. A medial incision was made on the head rostrally from above the eyes to the base of the neck. Cochleae were removed by breaking through the foreman magnum with rongeurs and carefully cutting away the bone surrounding the temporal bone. Formalin was perfused through the oval and round windows and the cochleae were stored in formalin until the decalcification process.

Decalcification and dehydration

Cochleae were decalcified by immersion in a 15% aqueous solution of tetrasodium Ethylenediaminetetra-acid (EDTA). The cochleae were placed in a series of alcohols of increasing concentrations (50%, 70%, 80%, 95%, and 100%) for 1 day each and then placed in 100% ether alcohol for another 1 day.

Embedding and sectioning

In a method similar to Schuknecht's (1953), the cochleae were placed in increasing concentrations (2%, 4%, 6%, 8% and 12%) of celloidin for 1 week each. After the bones had been in the 12% celloidin for 1 week, the top of the celloidin was hardened with chloroform, and excess celloidin was trimmed to create rectangular blocks. The blocks were mounted on cutting blocks so that vertical or horizontal sections could be cut with a sliding microtome knife (American Optical Company, model 860). Sections were cut at thicknesses of 20 microns, placed on numbered paper, and every fifth section removed for slide preparation. Sections were consecutively numbered beginning at the apex and proceeding toward the base or beginning at the lateral most aspect of the cochlea and proceeding medially.

Staining and mounting

The sections were stained with hematoxylin and eosin in a method similar to that described by Schuknecht (1953, 1974). The sections were rinsed in 50% alcohol and then distilled water and placed in Harris Hematoxylin for 25 minutes. They were rinsed in sequence in distilled water, acid alcohol until pink in color, distilled water, and then ammonia water until blue in color. The sections were then dipped into eosin solution for 30 seconds. The sections were moved through two changes of 95% alcohol solution and placed in a one-to-one alcohol/chloroform rinse. Finally sections were moved through a one-to-one terpineol/xylene rinse and mounted on glass slides. A mounting medium was used to place the section and the cover slip was pressed into place.

Organ of Corti description

Measurements of tangential cuts through the organ of Corti were made and placed along an axis on graph paper. Each cochlear turn was measured as the distance between the heads of the pillar cells along adjacent turns and were plotted on graph paper, producing a spiralgram. The organ of Corti was represented as a series of observations of OHCs and IHCs. Each series consisted of observations made from every fifth section indicated above. Each normal hair cell was recorded as a large open dot in its relative location within the box. Abnormal appearing hair cells were represented with filled dots. Absent hair cells were represented by x's. When the observations had been made, and the dots and x's placed in the proper location, a reconstruction of the cochlea appeared. Areas of missing hair cells were plotted in a histogram, termed a cytocochleogram.

Design

Animals in the nonexposed, early- and late-exposed groups were used for both ABR assessment and histology. The surgery only and histology only animals were used for structural evaluation of the cochlea and associated sensory components.

Experiment One

The purpose of this experiment was to assess the effect of noise-exposure at a particular GA on cochlear development. Two experimental questions were addressed: (1) Does noise exposure delivered to a 113 day GA fetus have an effect on the continuing development of the ABR? (2) Does the histologic integrity of the cochleae harvested at 136 days GA from the nonexposed and the early-exposed groups differ qualitatively?

To answer question one, only the nonexposed and early noise-exposed groups were considered. The ABR was recorded at the intervals designated above. Mean thresholds and latencies by GA were determined for each stimulus type and level. The ABRs recorded from the nonexposed group were compared to GA-matched, pre- and postexposure ABRs from the early noise-exposed group.

To address the second question, cochleae harvested at the 136 days GA from the nonexposed, early-exposed, surgery only and histology only groups were qualitatively described.

The purpose of the surgery only and histology only groups was to assess the effect of ABR stimulation and surgery on the cochleae by comparing them to the nonexposed group. Table 2-1 provides a synopsis of events that occurred to answer the questions in Experiment One.

Experiment Two

The purpose of this experiment was to determine whether intense noise delivered at an earlier GA creates different amounts of change in the auditory system than a noise exposure delivered at a later GA. The two experimental questions addressed were: (1) Does intense noise create different changes in the ABR recorded from in utero fetuses exposed at 113 days GA versus fetuses exposed at 130 days GA? (2) Do the cochleae harvested 5 days after noise exposure have a similar appearance when the exposure occurs at an early versus late GA?

The ABR data from the early and late noise-exposed groups were used to address the first question. Mean ABR thresholds and latencies from late noise-exposed fetuses were compared to the early noise-exposed group. Differences in the preexposure, postexposure and 48-hour recovery ABRs were assessed.

To answer question two, cochleae from early and late noise-exposed groups as well as from the histology only

Table 2-1. Experiment One Timeline.

	GA of Surgery	GA of Exposure	GA of ABR	GA of Histology
Early-exposed group	110	113	111, 113, 114, 123, 130, 136	136
Non-exposed group	110	NA	111, 113, 114, 123, 130, 136	136
Surgery only group	110	NA	NA	136
Histology only group	NA	NA	NA	136

group harvested at 119 and 136 days GA, were described. The noise-exposed cochleae were harvested of the fifth day after the cessation of the noise exposure.

Table 2-2 provides a synopsis of events completed to answer the ABR and histologic questions of Experiment Two.

Data Analysis

Identification of peaks and ABR thresholds were determined by two independent observers experienced in ABR recording techniques. Latency measurements of the vertex-positive peaks for clicks, and 2 kHz were recorded with one of the observers blinded to treatment condition. Differences in latency scoring were resolved by averaging the two latency values, if there was less than a 0.1 ms difference. Differences greater than 0.1 ms were resolved by reexamining the waveform. ABR thresholds for clicks, 2 kHz, 1 kHz and .5 kHz were defined as the lowest level at which a positive ABR waveform was identified.

Analyses of the ABR thresholds and latencies were completed using one-way analysis of variance for t-tests. Differences in the mean absolute latency values for clicks and 2 kHz, accounting for stimulus type, level, GA, age at exposure, and exposure condition, were simultaneously compared. Differences in the mean ABR threshold for clicks, 2 kHz, 1 kHz, and .5 kHz, accounting for stimulus type and

Table 2-2. Experiment Two Timeline

	GA of Surgery	GA of Exposure	GAs of ABRs Pre, Post, Recovery	GA of Histology
Early group (N = 6)	110	113	113, 114, 116	119
Late group (N = 6)	127	130	130, 131, 133	136

level were made for GA, age at exposure, and exposure condition were compared.

CHAPTER III
RESULTS AND DISCUSSION

Subjects

Thirty-four animals were utilized for this study, and 24 completed the protocol. Twenty-eight animals were instrumented for ABR measurements and grouped as follows: nine in the nonexposed group; thirteen in the early noise-exposed group; and six in the late noise-exposed group. Six animals were used only for histology, and were not instrumented for ABR.

Of the 11 preparations that failed to complete the protocol, there were three instances of bone oscillator failure in an otherwise healthy animal, one ewe death after surgery, two fetal deaths after surgery, and five abortions or early deliveries. One cochlea from each of three subjects was damaged during processing. These cochleae were excluded from histologic analysis and the contralateral cochleae, also available from these animals, were chosen for preparation.

Nonexposed Subjects

Normal ABR Development

The animals in this group were prepared surgically at 110 dGA, generated ABR measurements 1 day after surgery, followed a testing schedule indicated in Chapter II and were sacrificed on 136 dGA. The ABR morphologies, thresholds, and latencies showed rapid development from 111 to 136 dGA. Figure 3-1 shows the click-evoked ABR changes that occurred over time for a typical nonexposed subject. At 111 dGA, the ABR was present in all 22 ABR-instrumented subjects (nine from the nonexposed group plus 13 from the early noise-exposed group), who had undergone surgery at 110 dGA. The morphology of the waveform, although repeatable and indicative of a positive response at 41 dB nHL (the output limit of the bone vibrator without distortion), did not typically allow for the identification of peaks. Of the 22 subjects tested at 111 dGA, only one produced any repeatable peaks. A positive response at 111 dGA was characterized by a positive deflection in the waveform at around 2 ms or a negative drop that emerged at approximately 7-8 ms.

By day 116 dGA, ABR waveform morphology was more distinct. At this test date, eight of the

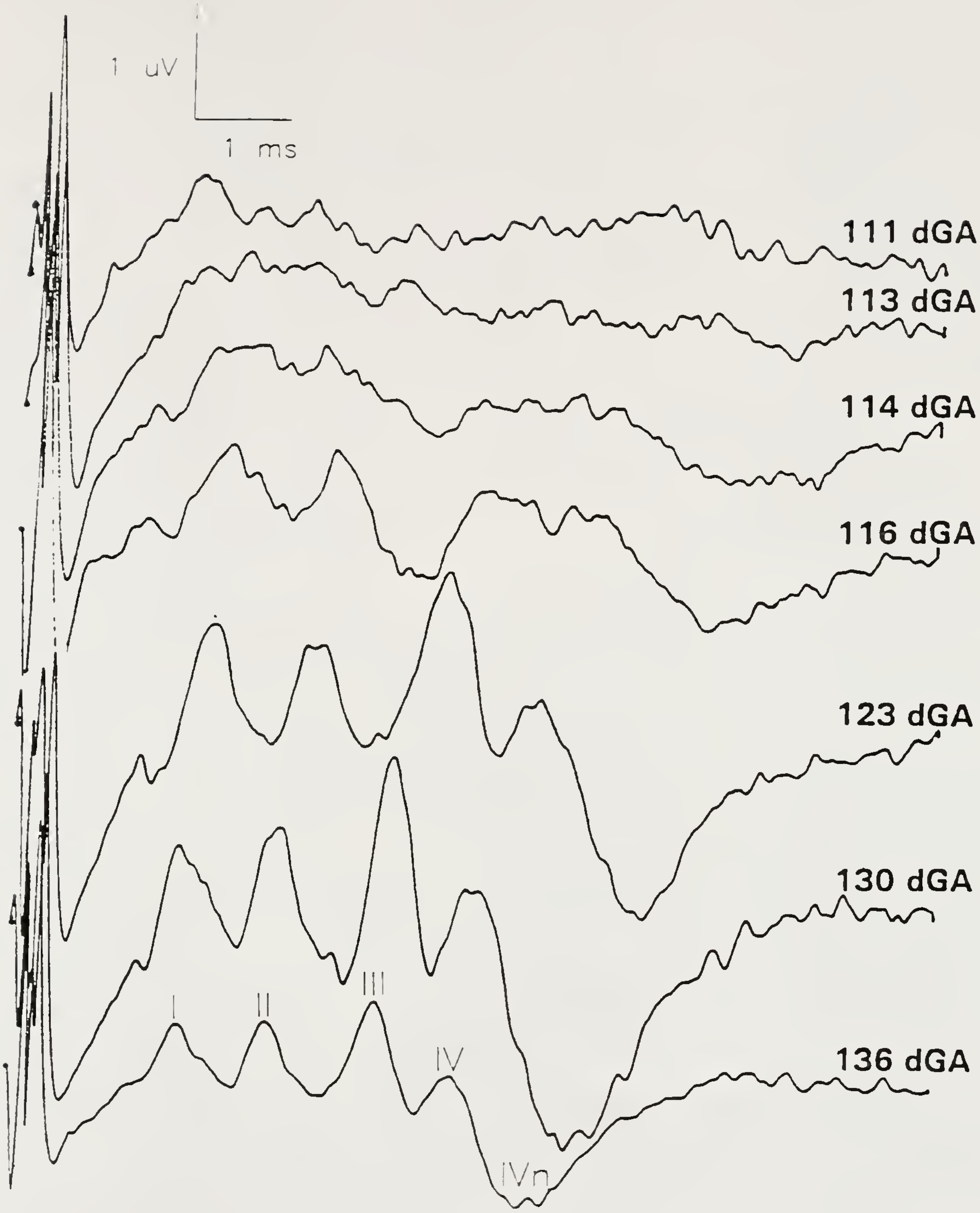


Figure 3-1. Click-evoked ABR waveforms recorded at 41 dB nHL from subject number 1018 over increasing GAs. The waveform for 136 dGA is displayed at one-half the amplitude of the others for purposes of plotting.

nine subjects in the nonexposed group produced reliable ABR waveforms with four vertex positive peaks. By this time the early noise-exposed group of animals had been exposed. The morphology of the ABR continued to improve up to 136 dGA, when the click-evoked ABR in fetal sheep was characterized by "four" peaks in the waveform. At 41 dB nHL, Wave I had a mean latency of 1.75 ms; Wave II, 2.61 ms; Wave III, 3.85 ms; and Wave IV, 4.59 ms.

The presence of "four" peaks were in agreement with observations of Wolfson et al. (1990), from exteriorized premature lambs but contradicted the "seven" peaks identified in lambs by Ashwal et al. (1984) and "five" peaks in fetuses by Woods and Plessinger (1985) and Woods et al. (1987). The morphologies of the fetal ABR waveform recorded in the current study and the fetal and lamb ABR waveforms recorded in the previous studies were similar. The differences in the number of waveform peaks can be explained by peak "labeling," with their peaks I-IV corresponding to peaks I-IV in the current study. The difference in the number of observed peaks in the studies by Ashwal et al. (1984), Woods and Plessinger (1985), and Woods et al. (1987) resulted from their labeling a "fifth" peak at approximately 5-6 ms in the Ashwal study and 7-8 ms in the Woods study, much later than the other peaks that typically constitute the ABR (Jewett and Williston, 1971).

The presence of repeatable waveforms at 111 dGA was several days earlier than the first response obtained from *in utero* fetal sheep at 117 dGA by the Woods' group (Woods and Plessinger, 1985). The difference in transducers used in these studies (a hearing aid receiver covered with rubber was used by Woods and a bone oscillator was used in the current study) may account for the time at which the ABR first emerged.

Wolfson et al. (1990) were able to identify four repeatable peaks in two exteriorized fetal sheep at 106 dGA. They used a bone oscillator to deliver the click stimulus. However, the fetus had been delivered by caesarian section and its ABR recording was obtained within 30 minutes after delivery. The premature lambs used in the Wolfson et al. (1990) study were sustained on liquid ventilation. Additionally, stimulus levels between the Wolfson and current study are difficult to compare because of methodological variations. Nevertheless, a comparison of the current findings to the Wolfson findings suggest that there are differences between the *in utero* and *ex utero* recording environments. Two possibilities for these differences are an impedance mismatch from the fluid-filled *in utero* environment to the air medium of the *ex utero* environment, which could alter middle ear function and influence the ABR. Also, the performance of the bone oscillator when surrounded by fluid clearly differs from its performance in air. Both factors may influence the morphology of the ABR because of stimulus intensity differences present at the inner ear.

Not only did the ABR waveform morphology change over time, but ABR thresholds also decreased rapidly. Figure 3-2 displays mean ABR thresholds over time for the four stimuli types for the nonexposed group. Means, standard deviations, minimum and maximum thresholds for all animals can be found in Appendix A, Section A. Recall that 0 dB nHL is referenced to human behavioral sensitivity with the bone oscillator placed on the skin overlying the mastoid. In contrast, the bone oscillator on the fetal sheep was applied directly to the skull. Thus, fetal ABR thresholds for 123 dGA and above were found at stimulus levels below audibility for human subjects.

Rapid changes in threshold occurred for all stimuli types from 111 dGA to 123 dGA. For example, during this period, the ABR threshold of the 2 kHz stimulus improved 38 dB. This improvement was the most remarkable change, but the responses to other stimuli showed similar magnitudes of change. The most rapid change in threshold (2 kHz) from 111 to 123 dGA was 3.17 dB/day.

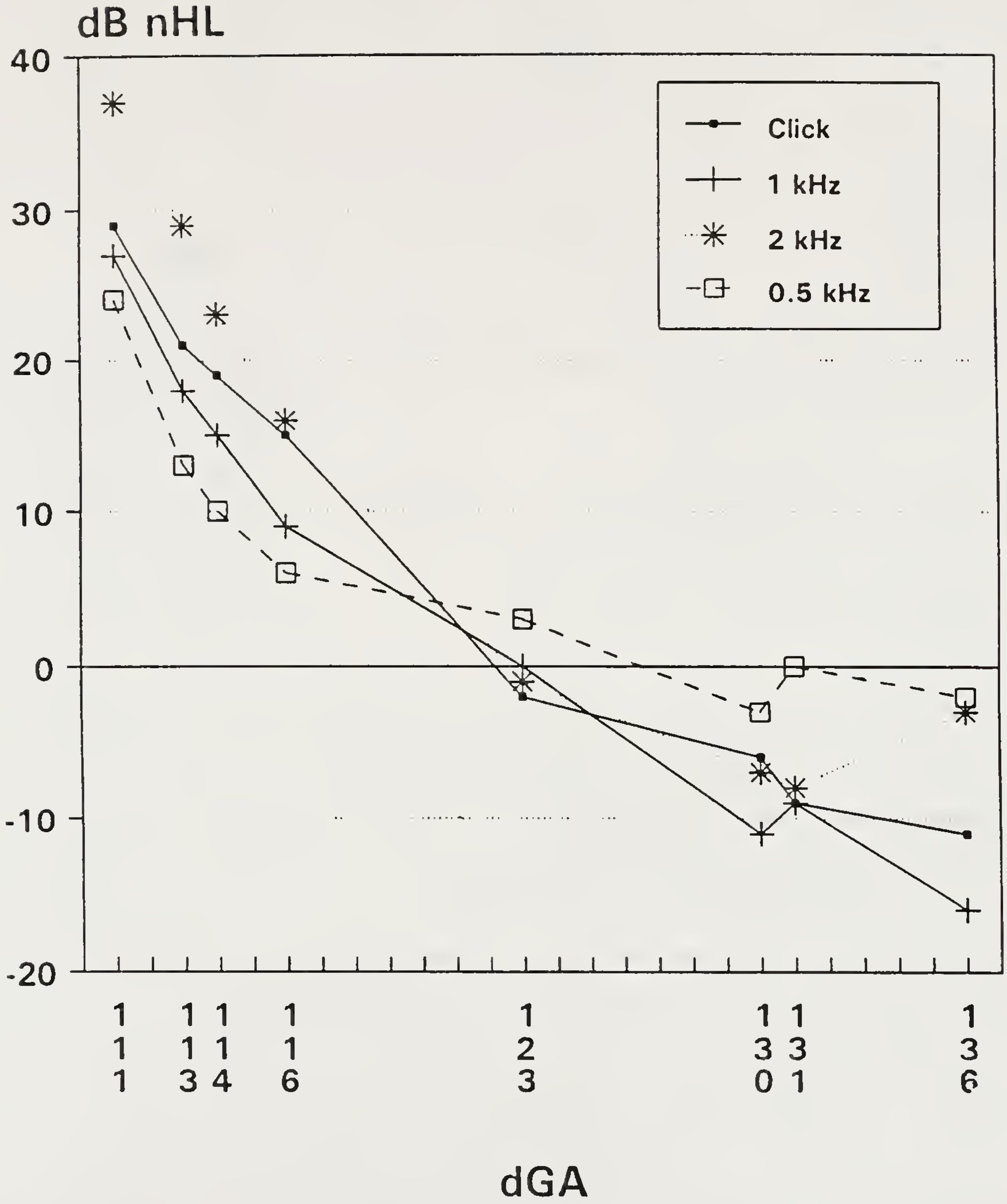


Figure 3-2. Mean ABR thresholds to clicks, 2, 1, and 0.5 kHz tone bursts recorded *in utero* from nonexposed fetuses.

Thresholds for 2 kHz continued to decrease between 123 and 136 dGA, but at a rate of approximately 0.15 dB/day.

At 111 dGA, thresholds for 0.5 kHz were 3 to 15 dB lower than thresholds for 1 kHz, 2 kHz and click stimuli and remained so until 123 dGA. At that time, thresholds for 0.5 kHz became poorer by 3 to 5 dB. At 136 dGA, 0.5 kHz thresholds were 2-15 dB poorer than thresholds for other stimuli. These differences may be related to developmental changes in bone-conducted ABRs. Yang et al. (1987) and Hooks and Weber (1984) found for infants that latencies to bone-conducted clicks (lower in frequency than air-conducted clicks) were not longer than adult latencies. The authors of these studies argued that a base-to-apex developmental gradient, known to occur postnatally in some animals and prenatally in humans and other animals, could result in shorter latencies for undeveloped ears. This same line of reasoning could apply to ABR thresholds. Lower thresholds to low frequencies compared to thresholds for higher frequencies could be a function of development of the place principle. Other factors may well have been involved, including frequency-specific changes in transduction properties at the bone oscillator-skull interface that favor signal efficiency early after placement to the skull, but which change as the skull grows. Clarification of these issues await further study.

The results for the earlier dGAs were in agreement with the basal to apical theory of cochlear development (Rubel and Ryals, 1983), i.e. the low frequencies are effective initially in the base and the place code moves apically during development. Conversely, during maturation, the ABR thresholds for low frequency stimuli are not as sensitive as clicks or higher frequency stimuli because of the travel time along the basilar membrane needed for the low frequency stimuli (Kiang and Moxon, 1974; Davis and Hirsh, 1979).

Of the remaining stimuli types in the current study, the click and 2 kHz tone burst thresholds were the most similar. Presumably, the 2 kHz tone burst elicited responses from fibers more basal than the 1 kHz and 0.5 kHz tone bursts and may be closer to fiber locations which contributed to the click responses. The low thresholds for the 1 kHz stimulus at the later GAs may have been related to the resonance of the bone oscillator. Refer to Figure 2-1 for the spectra of the 1 kHz tone burst.

As one example, the 130 dGA mean ABR latency-intensity (LI) functions evoked from the nonexposed fetuses with the click and 2 kHz tone burst stimuli are shown in Figure 3-3. Refer to Appendix A, Section B, for the means, standard deviations, minimum and maximum latencies for Waves I-IV, the IVn trough, and amplitude for all animals. The use of decreasing 20 dB steps in intensity and the resulting three data points per stimulus type gave the appearance of an

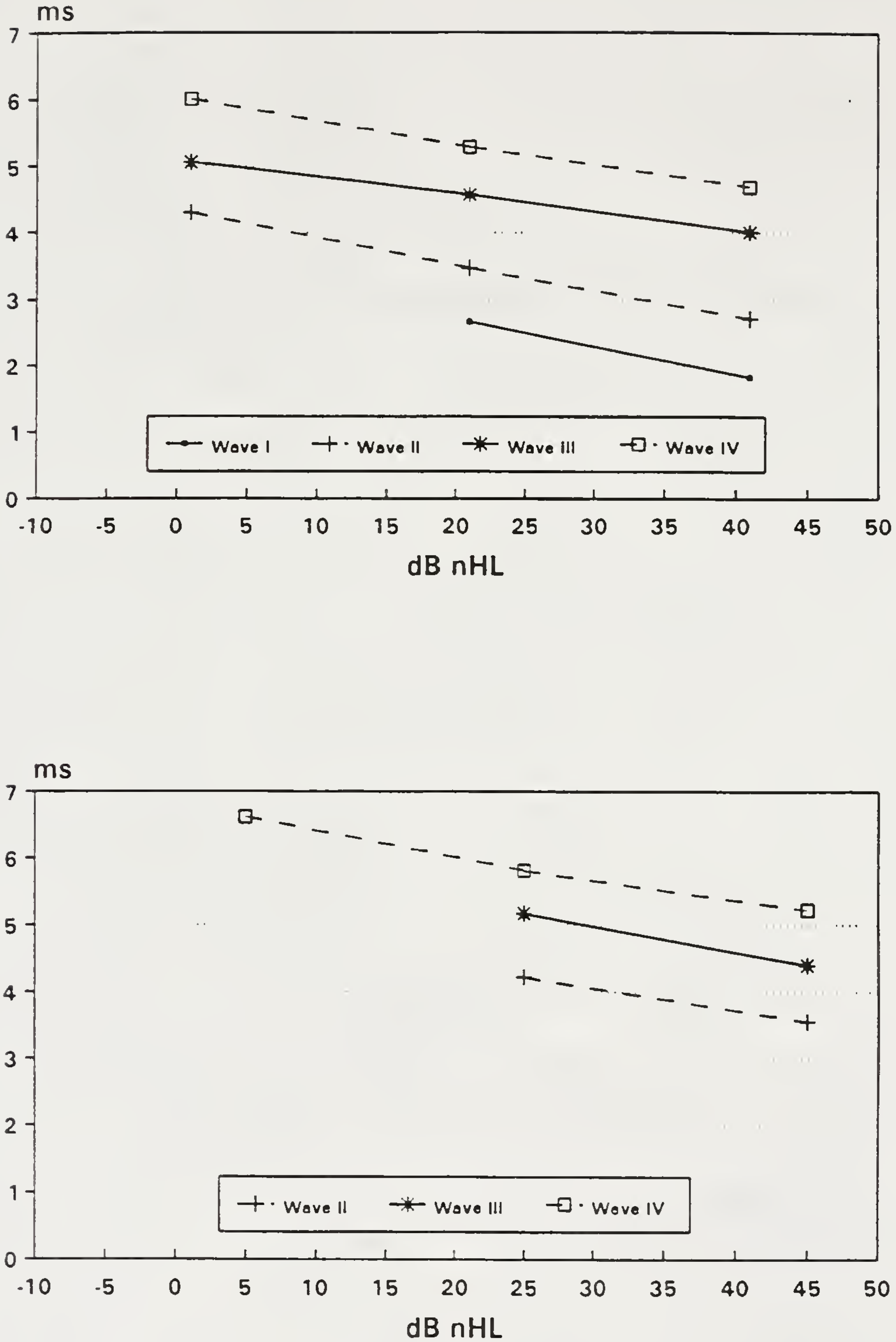


Figure 3-3. Mean ABR latency-intensity functions for 130 dGA nonexposed fetuses: A. click; B. 2 kHz tone burst.

atypical linear function. However, the slope of the LI function was similar for all waves for both stimuli types. All latencies decreased between 0.5 and 0.75 ms per 20 dB decrease in intensity. Latency shifts of these magnitudes were consistent with the literature from human ABR latency-intensity functions. For example, Schwartz and Berry (1985) reported 0.4 ms/10 dB and Rosenhamer (1981) reported 0.01 ms/dB, which are equal to 0.8 and 0.2 ms/20 dB, respectively.

The 2 kHz Wave I was not identified because of the length of stimulus artifact. Also, the 2 kHz peaks that are identifiable (Waves II, III, and IV) had longer latencies than the click-evoked peaks. Once again, these longer latencies were related to the stimulus travel time on the basilar membrane.

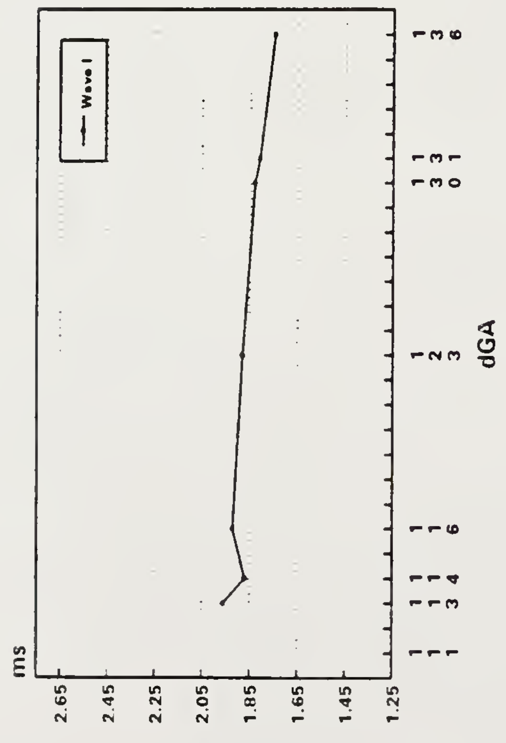
Not only did thresholds decrease rapidly over time, but latencies decreased during development from 111 dGA to 136 dGA. In Figure 3-4, the mean click-evoked Wave I-IV latencies for two intensity levels (41 and 21 dB nHL) are displayed. Overall, latencies for the four identifiable peaks decreased over increasing GA.

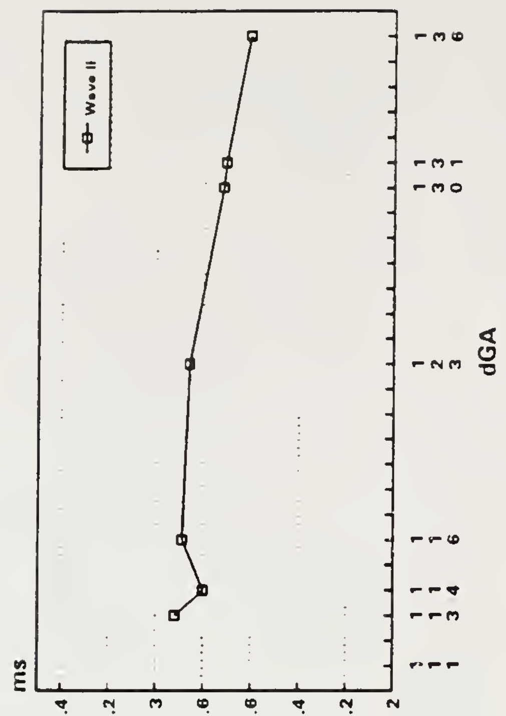
These decreases in the latencies are mostly related to increased myelination, increased axon diameter, and the resulting improved neural synchronization (Shah et al., 1978; Krumholtz et al., 1985). At both intensity levels, latencies of Waves I and II decreased slightly, but much

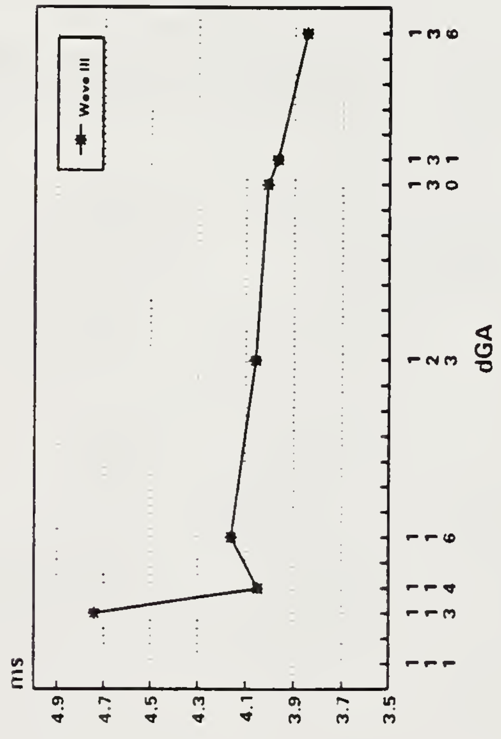
Figure 3-4. Various GA's mean nonexposed click-evoked Waves I, II, III and IV latencies at two intensities:

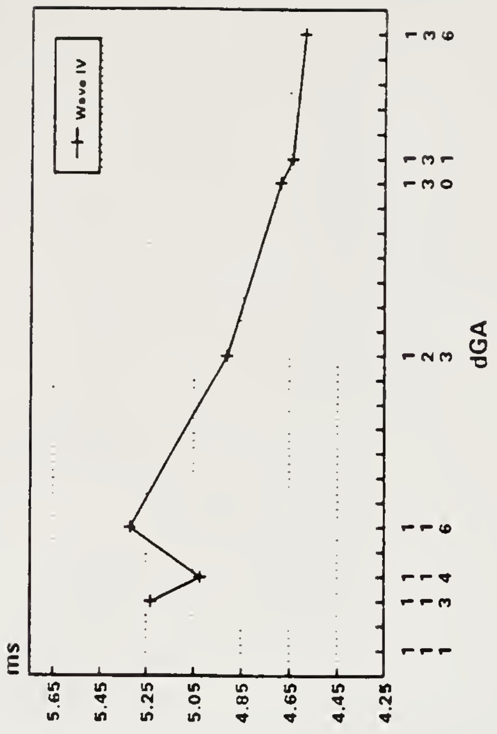
A. 41 dB nHL click

B. 21 db nHL


ms
2.65
2.45
2.25
2.05
1.85
1.65
1.45
1.25
Wave I
111
113
114
116
123
130
131
136
dGA


ms
3.4
3.2
3
2.8
2.6
2.4
2.2
2
Wave II
111
113
114
116
123
130
131
136
dGA


ms
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
Wave III
111
113
114
116
123
130
131
136
dGA


ms
5.65
5.45
5.25
5.05
4.85
4.65
4.45
4.25
Wave IV
111
113
114
116
123
130
131
136
dGA

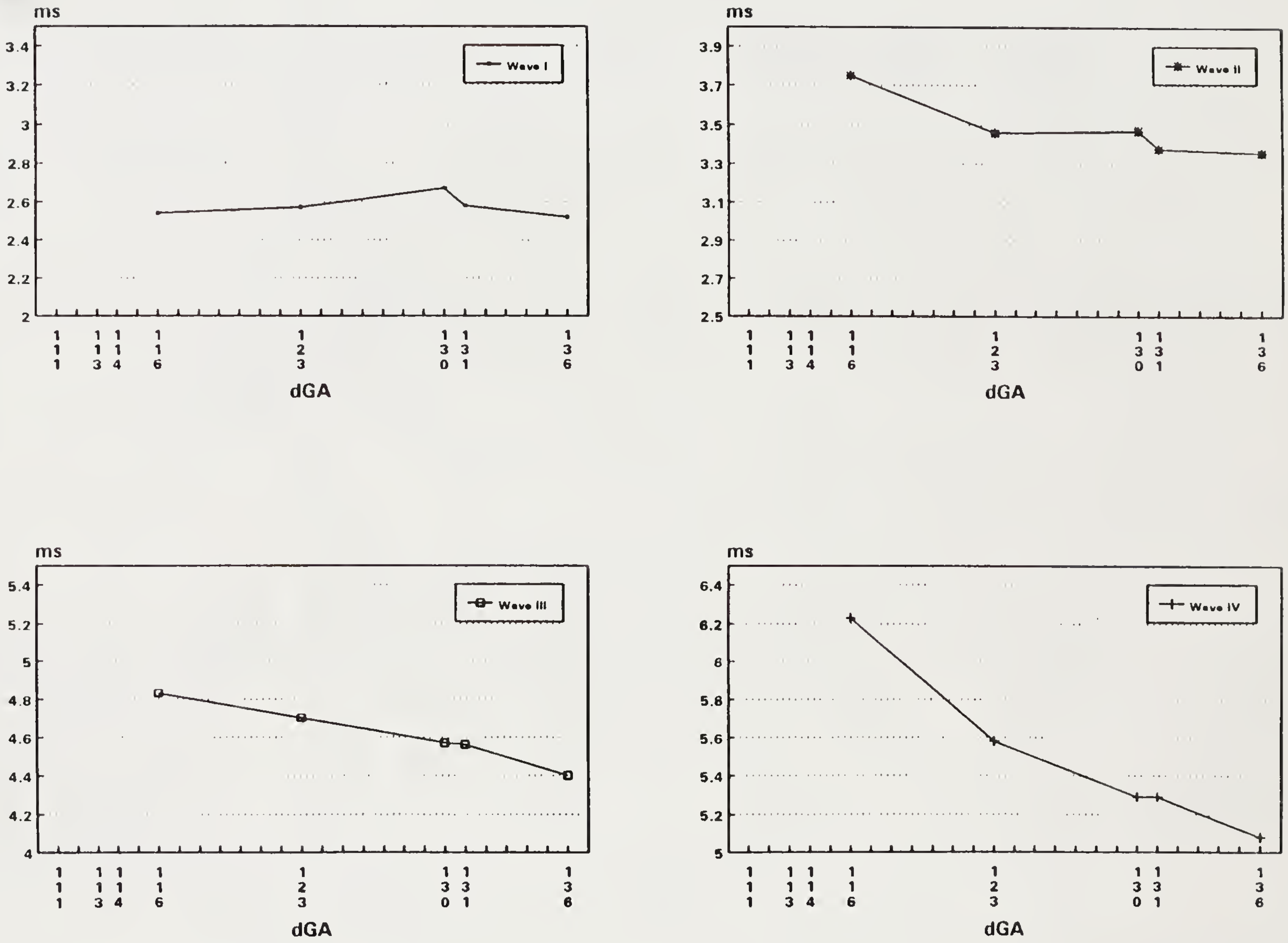


Figure 3-4. Continued.

greater changes were observed for latencies of Waves III and IV. These findings were in agreement with Wolfson et al.'s study and a human developmental study by Salamy and McKean (1976). Salamy and McKean hypothesized differential rates of maturation between the peripheral and central auditory pathways.

Another factor contributing to the latency decrease could be related to development of place specificity. In a study investigating the effects of forward masking on human Waves I to V, Zapala et al. (1992) concluded that Wave V is more biased by apical influences while Wave I reflects more basal events. Following the base to apex developmental course, Wave I then should initially represent the more developed portion of the cochlea and consequently reflect fewer changes. If the cochlea was continuing to mature, later waves (III and IV in the sheep) would be expected to improve in latency as the apical portions of the cochlea continued to develop.

A third consideration for differential rates of change in peak latencies was the function of the supposed generators for Waves I and II (the distal and proximal ends of the cochlear nerve, respectively (Möller et al., 1981). If the cochlea and the cochlear portion of the VIIIth cranial nerve were at least partially functional, as they must be for the ABR response to occur, then they had the least ability to have shown improvement. In other words, because the Wave I latency was short when it was first identified, it was difficult for it show as great a decrease in latency as the later occurring waves.

Description of Normal Cochleae

The sheep cochlea was striking in its unusual shape. Most mammals possess a conically-shaped cochlea with scalae, larger at the base and smaller at the apex. The cochlear turns revolve symmetrically around the modiolus and the axis of the modiolus remains in about the same plane (Suga and Hattler, 1970; Harrison and Hunter-Duvar, 1988).

Basilar membrane length varies with species. For example, in humans, the length is roughly 35 mm and there are 2 1/2 turns, whereas in chinchillas, there are 3 3/4 turns (Yost & Nielsen, 1985).

Figure 3-5 is a photograph of the opened cochlea obtained from a 4 to 5 week old lamb. In the fetal sheep, the organ of Corti consisted of 2 turns (Figure 3-5), but with a very long (approximately 8 to 9 mm) basal turn compared to the overall length (approximately 18 to 19 mm). The axis of the modiolus changes abruptly between the base and the middle turn. In one instance (Figure 3-6), a cross-sectional view of the organ of Corti occurred on the same serial celloidin section as a view from a superior aspect of the organ of Corti. This curiosity was produced by a tilt in the modiolar axis that occurred between the base and the

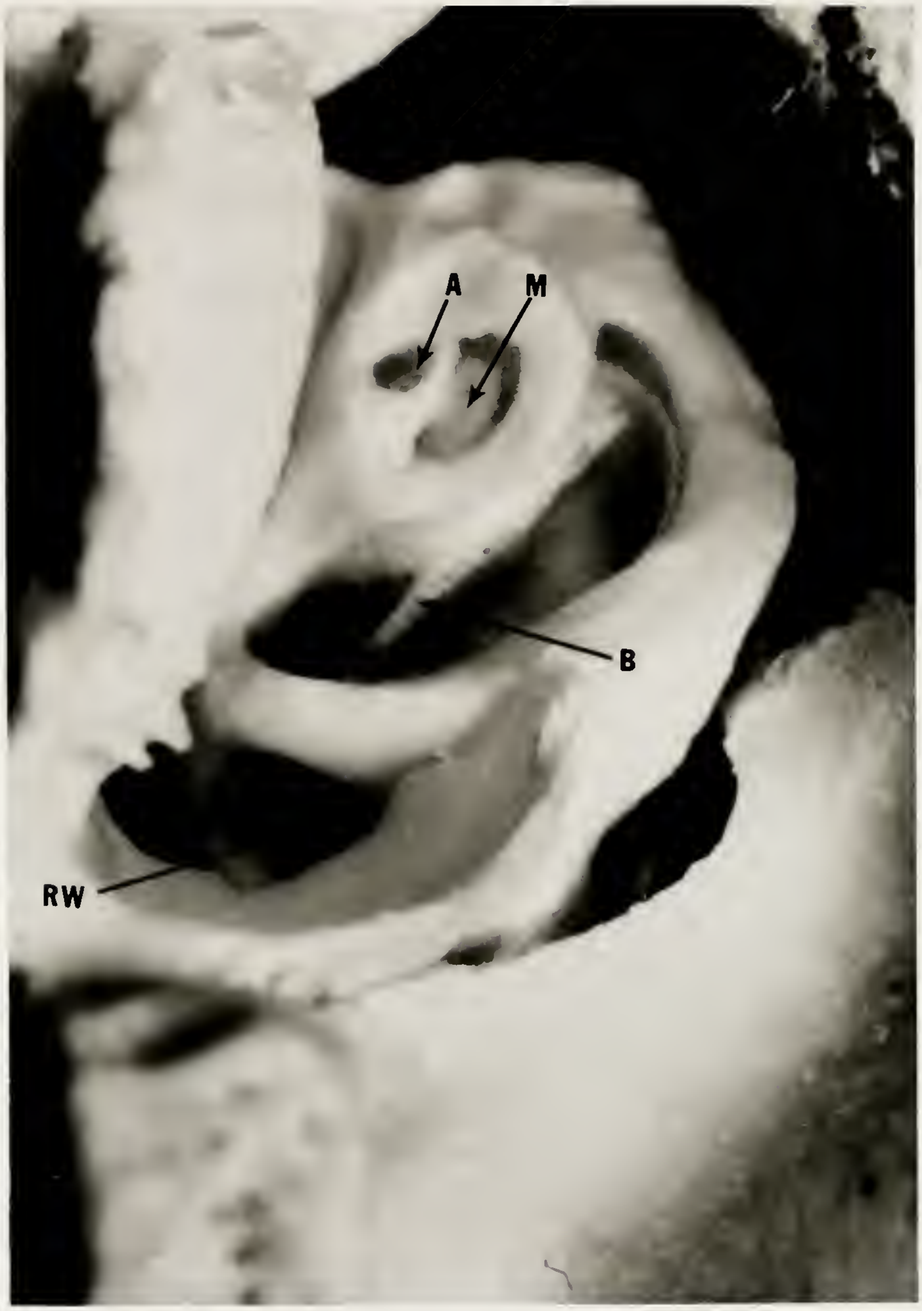


Figure 3-5. Photograph of the bony labyrinth of the right ear from a 4 to 5 week old lamb. RW, round window; B, base; M, middle; A, apex. Note the 90° rotation of the osseous spiral lamina between the base and middle turn.

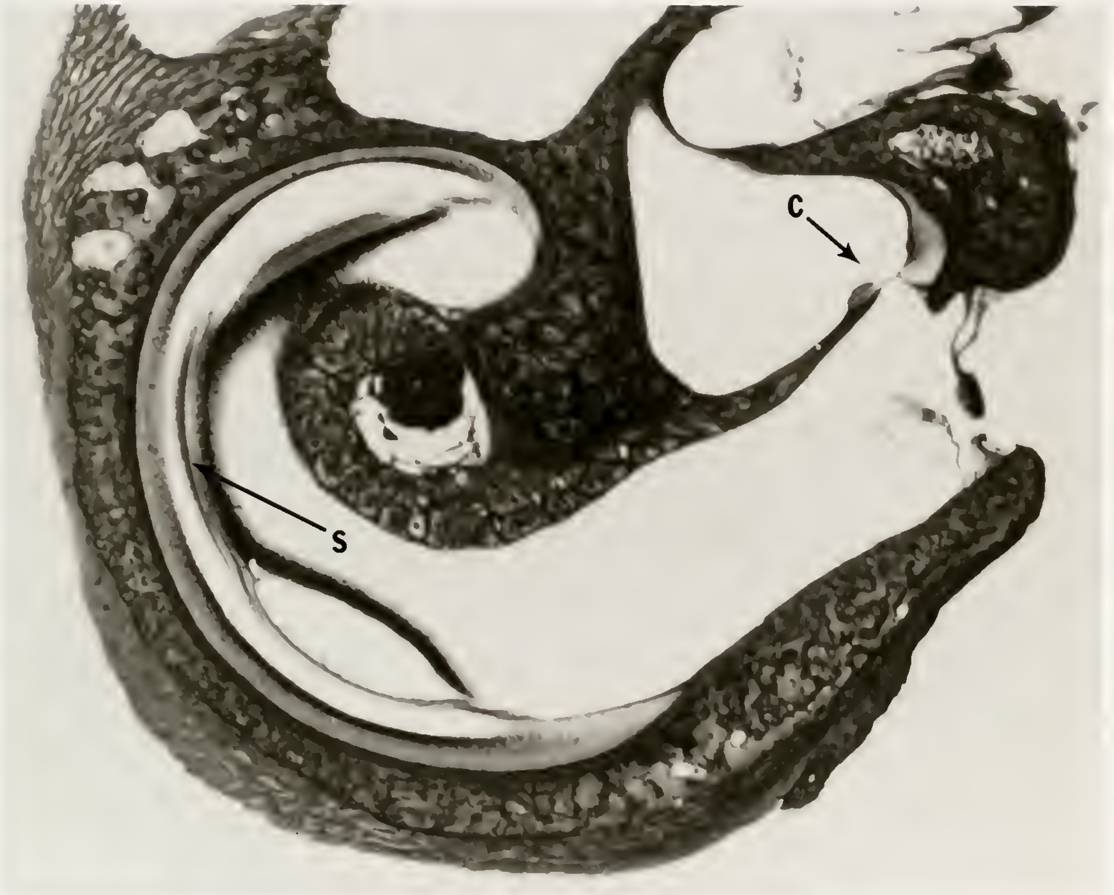


Figure 3-6. Cross-sectional view (C) and superior view (S) of the organ of Corti on the same serial celloidin section obtained from Subject 1011. The basilar membrane rotates 90 degrees during the first turn. See Figures 3-7A and 3-7B for greater magnification of these areas.

first turn. Figure 3-7 shows higher magnifications of the same two areas, a lateral view of the organ of Corti and a superior view of the organ of Corti.

The unusual shape of the fetal sheep cochlea is illustrated in the cochlear reconstructions. Figure 3-8 shows the "tear-drop" shape obtained for all fetal sheep subjects. An adjustment to the graphic reconstruction method used for other species (Guild, 1921; Schuknecht, 1953) had to be made. This adjustment was the reconstruction of the oval shape around the axis of the modiolus by measuring and plotting the distance between the pillar cells, instead of using a compass to construct semicircles. The reconstruction still was not exact, however, because the tilt in the modiolar axis could not be measured and illustrated from this type of preparation.

Because of the bend in the modiolus of the fetal sheep cochlea, there was difficulty in reconstructing the anatomy by the traditional serial sectioning method. It was impossible to complete a preparation without artifact caused by rotation of the plane in some sections. In most species, a consistent mid-modiolar plane of section is sought. However, when the fetal sheep cochlea was blocked in the proper position for cutting the basal sections, the apical portions were not mid-modiolar cuts. The opposite held true for obtaining a favorable cutting position for the apex.

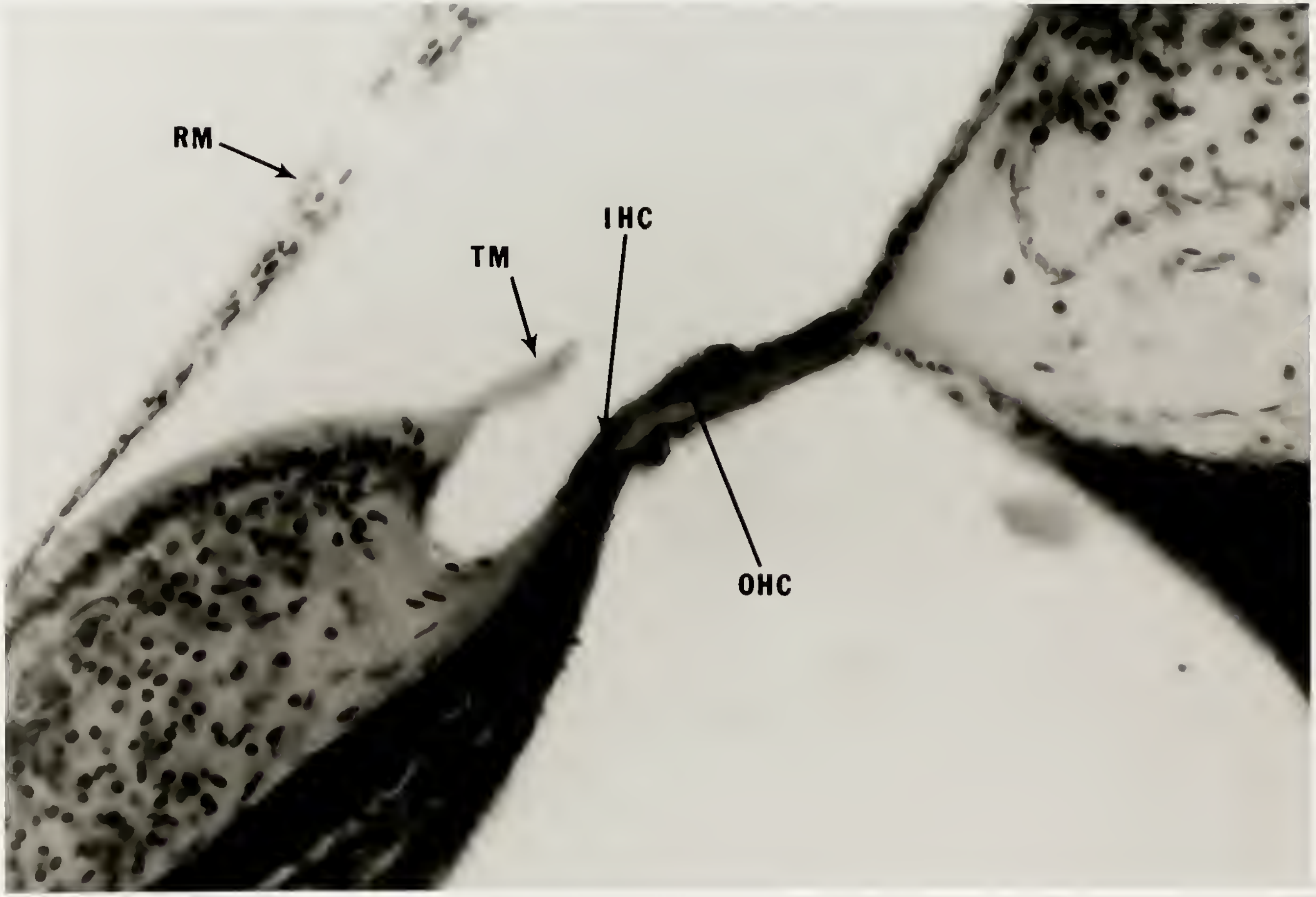


Figure 3-7. Magnification of two aspects of the organ of Corti seen on the same serial colloidin section.

A. Cross-sectional view. The IHCs, OHCs, tectorial membrane, Reissner's membrane, tunnel of Corti are all visible. RM, Reissner's membrane; TM, tectorial membrane; OHC, OHC nuclei; IHC, IHC nuclei.

B. Superior view looking down on IHCs and OHCs. Stereocilia on the OHCs are visible.

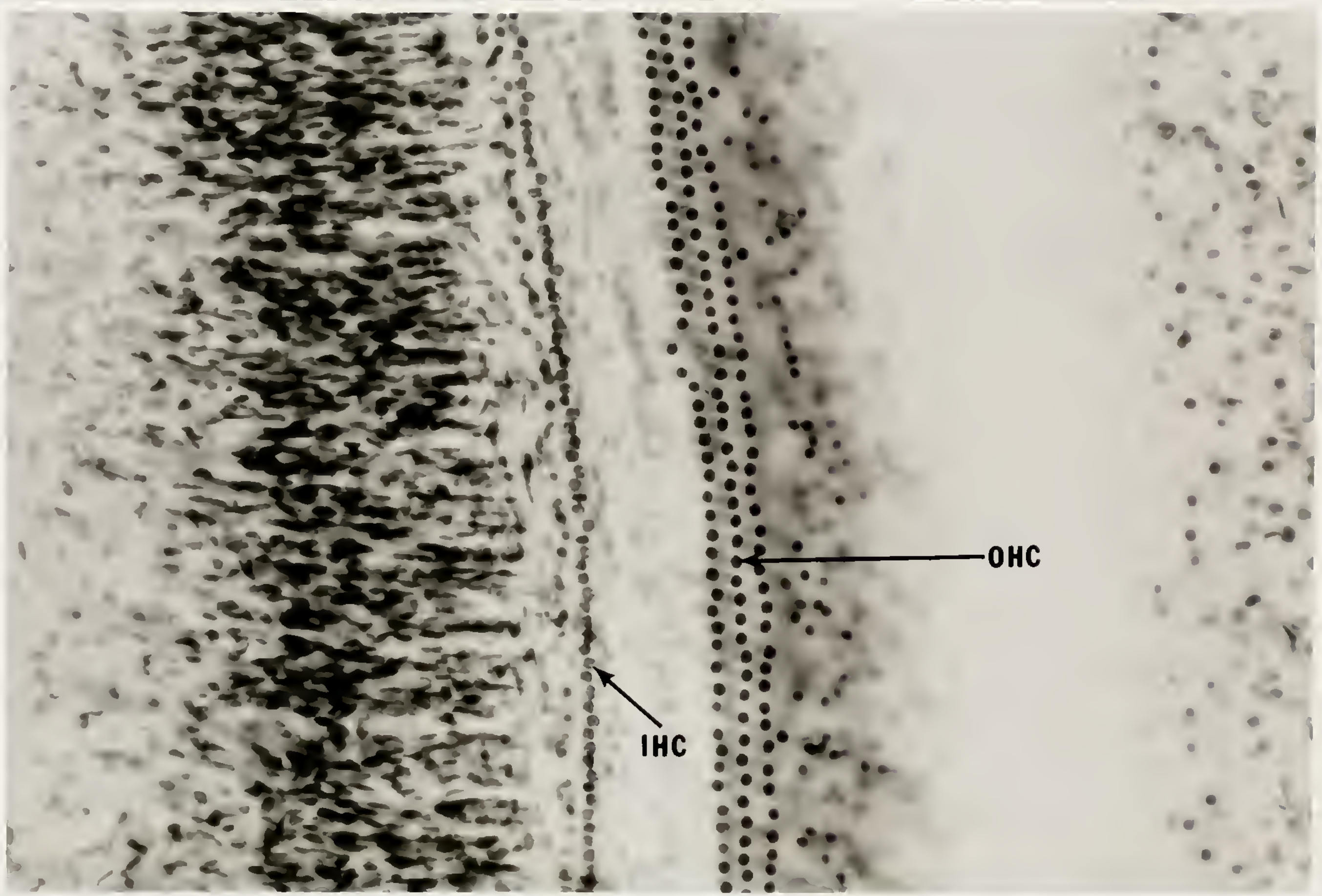
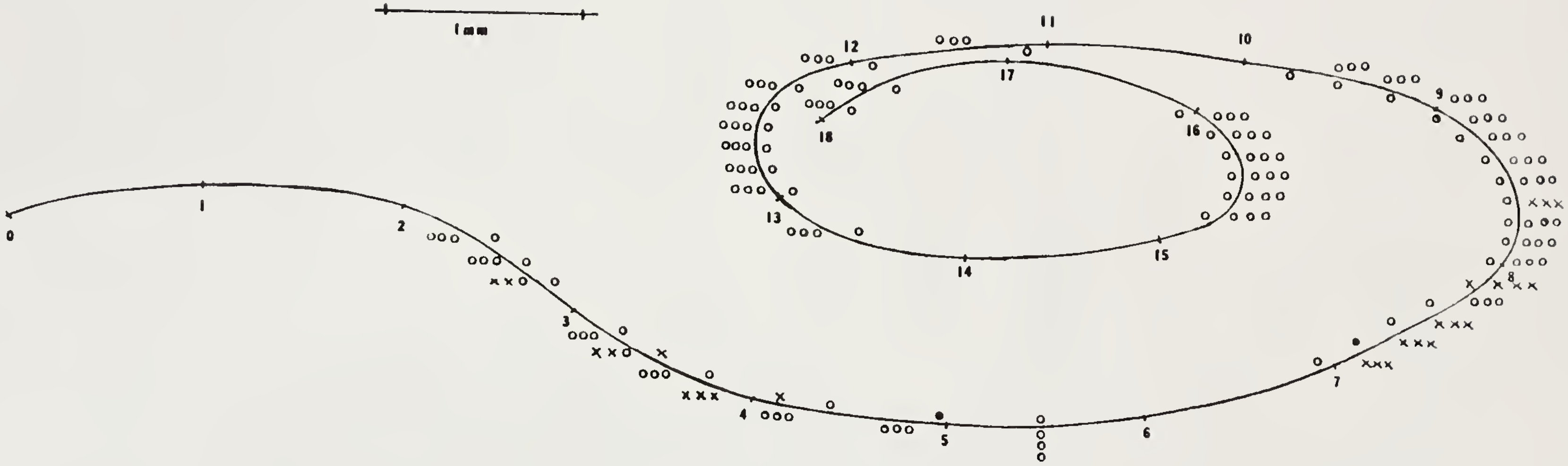


Figure 3-7. Continued.

Figure 3-8. Cochlear reconstruction for left ear of Subject 1006N, a 136 dGA nonexposed animal that was not instrumented for ABR.


1 mm
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

When the apex was optimally cut, the base exhibited cutting artifact.

Experiment One

Developmental Effect on the ABR

The purpose of Experiment One was to determine whether a noise exposure delivered to a 113 dGA fetus has an effect on the continuing development of the ABR.

Thresholds

Figure 3-9 illustrates mean ABR thresholds for all four stimulus types for the nonexposed and early exposed animals at various GAs. Means, standard deviations, minimum and maximum thresholds for all animals can be found in the Appendix, Section A. Following cessation of the noise exposure on 114 dGA, thresholds for the early noise-exposed group for all stimulus types were comparable to the nonexposed group. Thresholds for the early noise-exposed group did not increase on 114 dGA, immediately after the noise exposure. In other words, there was no threshold shift. This finding was not surprising since the preexposure thresholds were already high. For example, the mean preexposure click-evoked threshold was 21 dB nHL (which was close to the output limit of the ABR unit) and the mean postexposure threshold was 19 dB nHL.

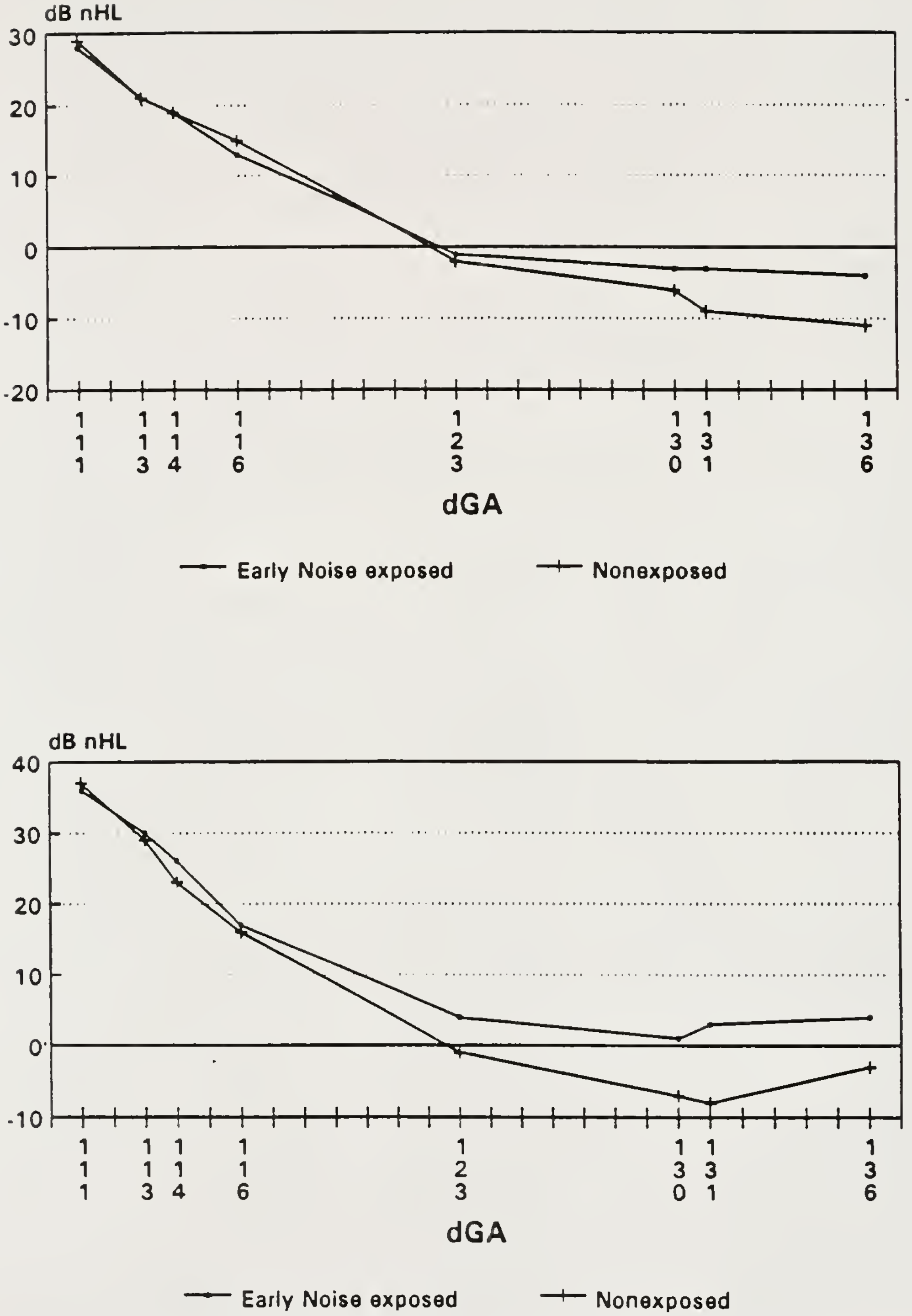
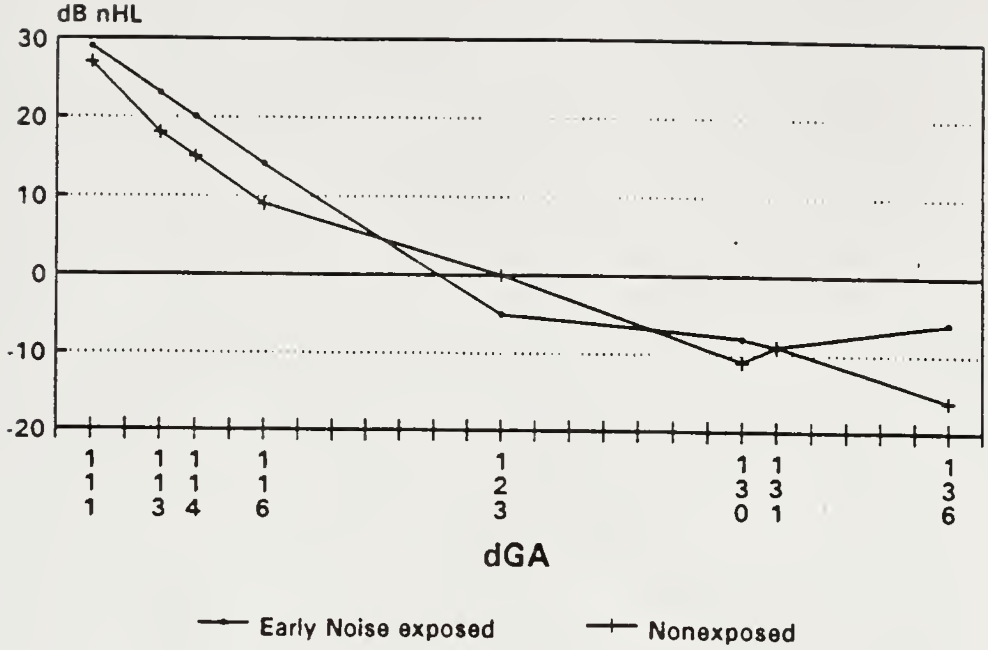


Figure 3-9. Mean ABR thresholds for the nonexposed and early exposed animals at various GAs. The 16-hour noise was presented at 113 dGA for the early noise-exposed group. A. Click; B. 2 kHz; C. 1kHz; D. 0.5 kHz.



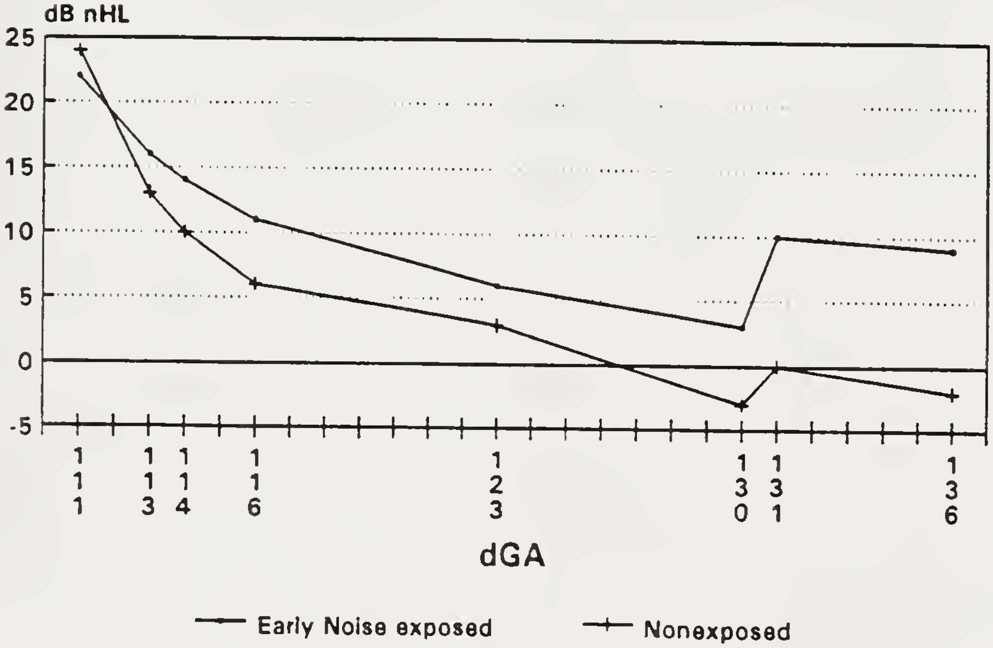


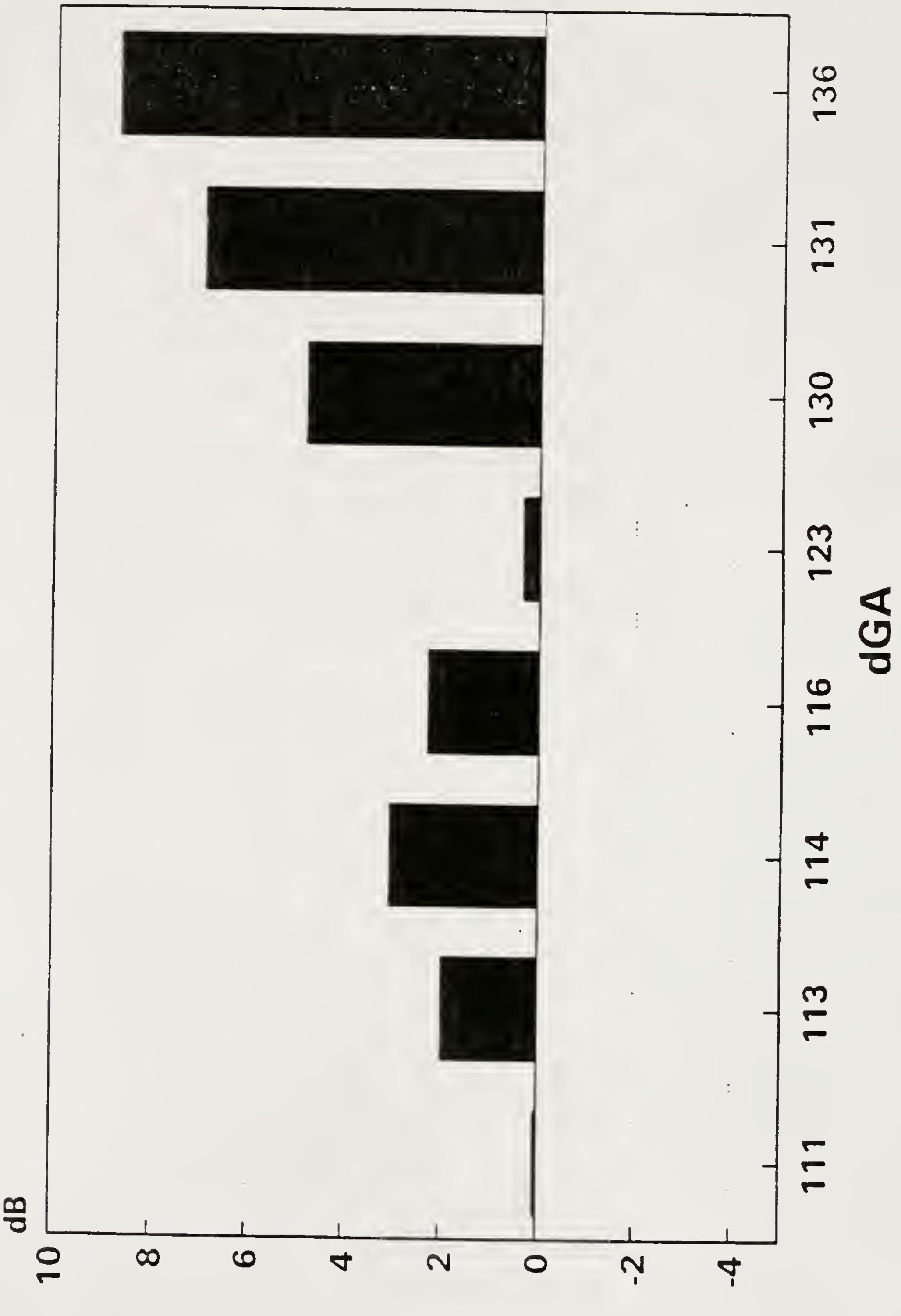
Figure 3-9. Continued.

In contrast to the earlier GAs, the mean nonexposed thresholds (after 116 dGA) were more sensitive than the thresholds of the early noise-exposed group. An exception is the threshold of the 1 kHz stimulus on dGA 123. At later GAs, a trend toward separation between the two groups appeared. Figure 3-10 illustrates threshold shift between the early-exposed group and the nonexposed group for all stimulus types for various GAs. Recall that the noise exposure occurred immediately before the 114 dGA measurement. Threshold shift was greater at 130, 131 and 136 dGAs. These results indicated that while there was little immediate threshold effect after the noise exposure on 114 dGA, the ABRs for the early noise-exposed animals recorded 2 weeks or more subsequent to the noise exhibited higher thresholds than nonexposed animals.

Figure 3-11 depicts the mean ABR thresholds for all stimuli at 130 dGA for the nonexposed, early and late noise-exposed groups. This day is a preexposure measurement for the late noise-exposed animals. A one-way analysis of variance revealed a significant difference among the thresholds of the three groups with an attained p-value of 0.0093.

These findings contradicted the Dunn et al. (1981) fetal sheep study, in which they reported no ABR threshold differences between a group of control animals and a group

Figure 3-10. Mean threshold differences between early noise-exposed and nonexposed animals for all stimulus types for various GAs. Each data point represents average of the early noise-exposed threshold minus the nonexposed threshold for the click, 2 kHz, 1 kHz and 0.5 kHz stimuli or threshold shift. Noise exposure for the early noise-exposed group occurred immediately before the 114 dGA measurement. T-tests for 130 dGA and 136 dGA revealed that threshold shifts were significantly different from zero with p-values of less than 0.05 and 0.01, respectively. Thresholds for 131 dGA were not significantly different from zero.


dB
10
8
6
4
2
0
-2
-4
111
113
114
116
123
130
131
136
dGA

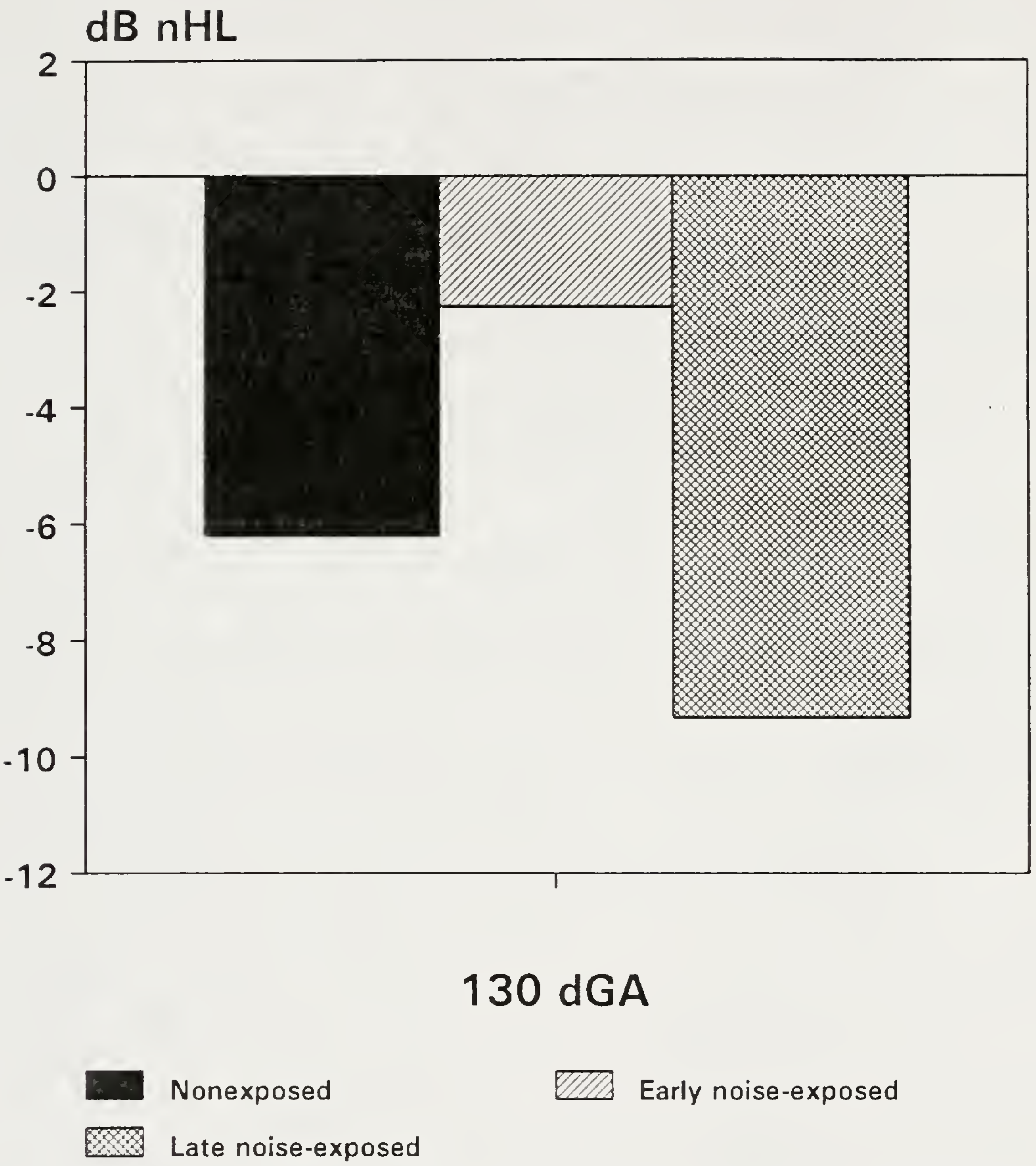


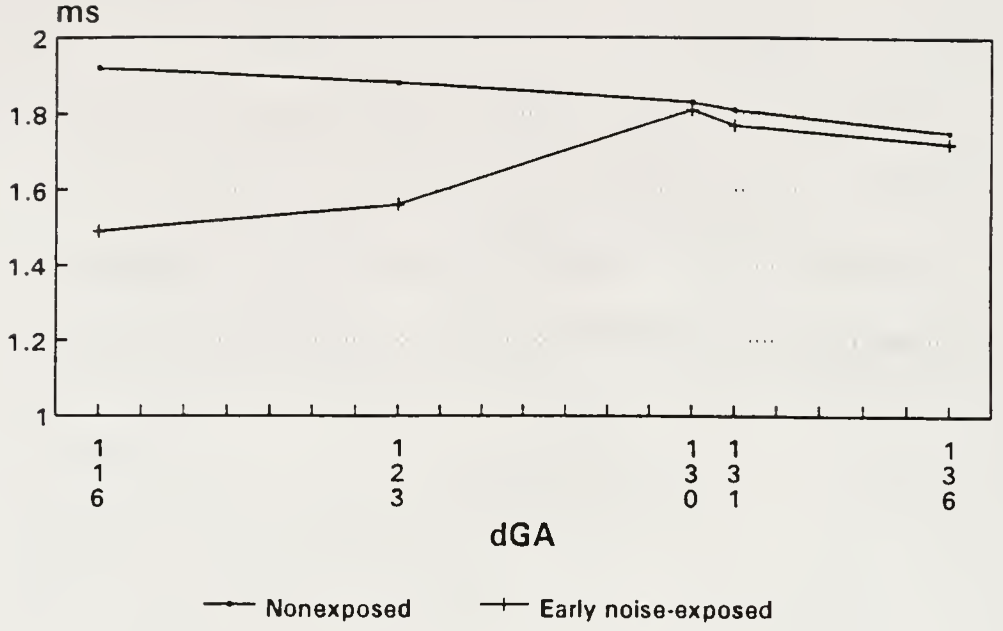
Figure 3-11. Mean ABR thresholds for all stimuli at 130 dGA. Analysis of variance revealed thresholds among the nonexposed, early- and late-exposed groups to be different with an attained p-value of 0.0093.

of lambs who had been prenatally exposed to 130 dB SPL 4 hours/day for 5 days a week for several months. However, because the results of the current study document shifts in ABR following noise exposure, it has more relevance to the human studies by Daniel and Laciak (1982) and Lalande et al. (1986). They found an increased risk of hearing loss in children whose mothers were occupationally exposed to hazardous noise. In the Lalande study, there was a significant increase in the children's risk of hearing loss at a frequency of 4000 Hertz (Hz) when the exposure involved a strong component of low-frequency noise. These studies have been criticized for methodological shortcomings (Henderson et al., 1993), such a lack of a matched control group. Nontheless, the results of the current study show that it is possible for an exogenous intense exposure to have some effect on hearing sensitivity *in utero*.

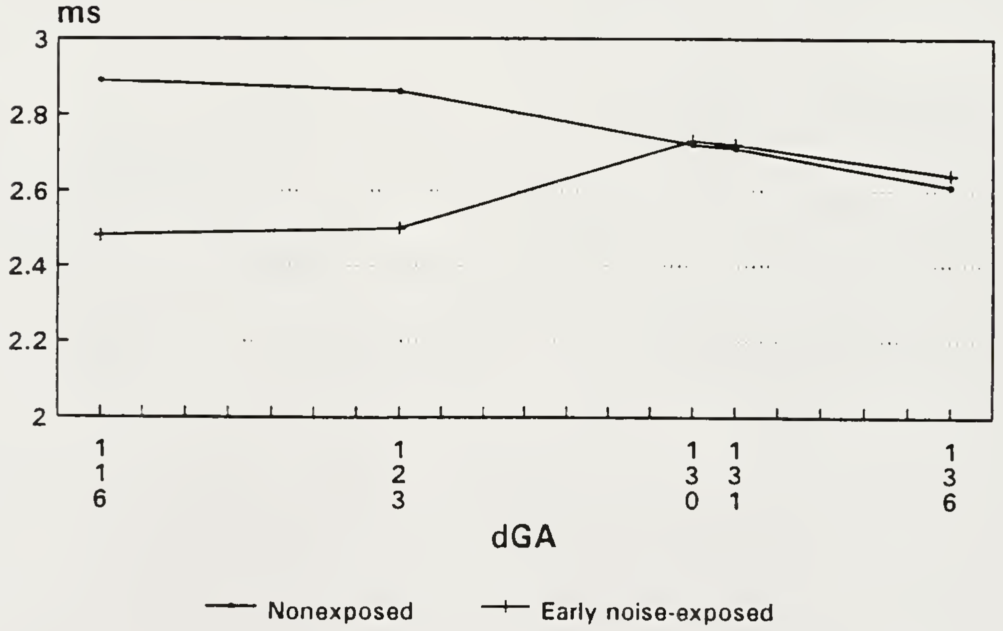
Latencies

Figure 3-12 contains the mean latencies for the 41 dB nHL click-evoked Waves I, II, III, and IV for nonexposed and early noise-exposed groups at various GAs. Refer to the Appendix, Part B, for the means, standard deviations, minimum and maximum latencies for Waves I-IV, the IV N trough, and amplitude for these animals. At 116 and 123 dGA, Waves I through IV mean latencies were shorter for the early noise-exposed group than for the nonexposed group.

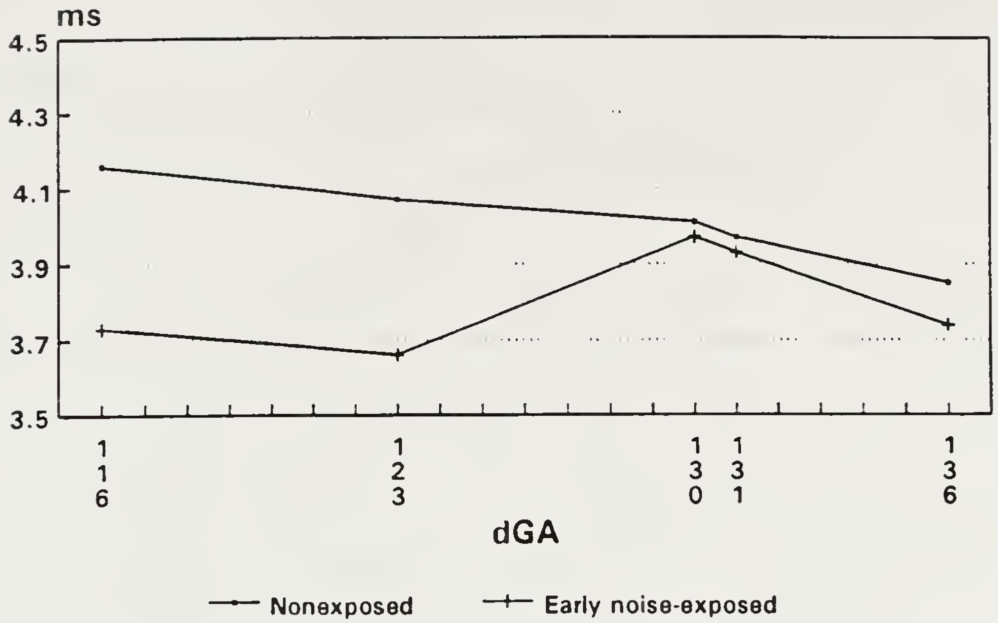
Figure 3-12. Mean ABR click-evoked latencies at 41 dB nHL for various GAs. Differences between the nonexposed and early-noise exposed groups at 116 and 123 dGA are not significant. A. Wave I; B. Wave II; C. Wave III; D. Wave IV.



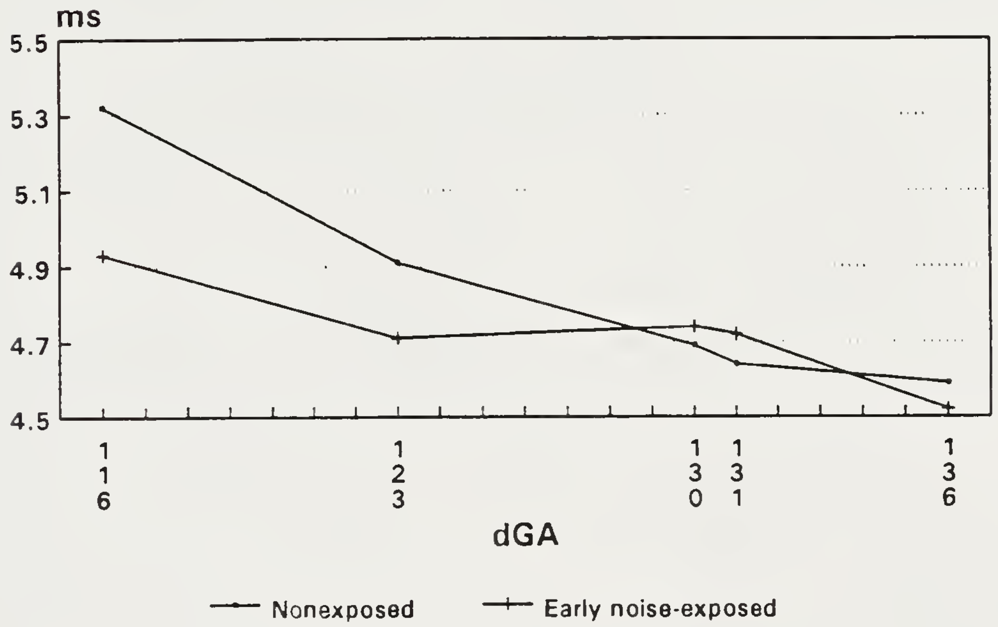
Wave I



Wave II



Wave III



Wave IV

Figure 3-12. Continued

For 130, 131, and 136 dGAs, this trend reversed and the latencies for the exposed and nonexposed groups were nearly identical.

Previous adult studies of NIHL and the ABR described increases in latencies following noise exposure. In humans with known hearing levels and a positive noise exposure history, many studies (Coats and Martin, 1977; Keith and Greville, 1987; Yamada et al., 1979) reported that high frequency hearing loss prolonged Wave I latency greater than Wave V latency. Jerger and Johnson (1988) reported that human Wave V latency increased as high-frequency hearing loss increased beyond 50 to 60 dB. In this study, a few days following noise exposure, Waves I through IV latencies were no longer for the noise-exposed group.

Experiment Two

Early Versus Late Exposure Effects on the ABR

The purpose of this experiment was to determine whether intense noise delivered to an earlier GA (113 dGA) fetus created a change in the auditory system which differed from a noise delivered to a later GA (130 dGA) fetus.

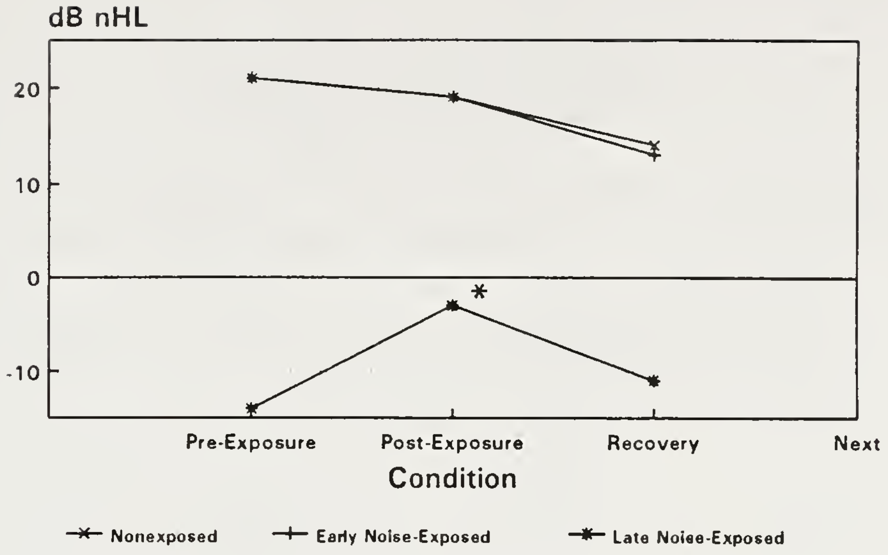
Thresholds

Mean preexposure, postexposure and recovery thresholds for the early and late noise-exposed groups, as well age-matched measurements for the nonexposed animals, can be found in the Appendix, Section A. Figure 3-13 illustrates mean thresholds for these three groups for the four stimulus types. The late noise-exposed group showed a postexposure increase in threshold followed by a decrease in threshold referred to as recovery for all stimuli. The increase in average threshold (7 dB) to click stimuli between the pre-exposure and postexposure recordings was significant with an attained p-value of 0.0062. Thresholds evoked by the 2 kHz, 1 kHz and 0.5 kHz tone bursts showed the same pattern of increased thresholds postexposure followed by a recovery decrease in threshold, but these postexposure increases were not significant.

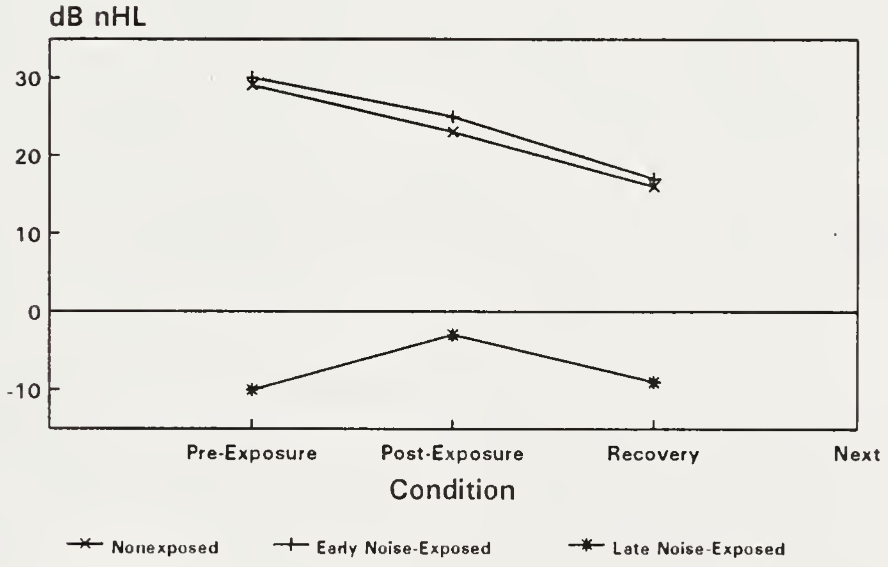
These findings are consistent with other NIHL studies, using other species, and indicate a small ABR threshold shift to click stimuli after noise exposure (Attias et al., 1990; Hildesheimer et al. 1991). Attias et al. reported a temporary increase in rat ABR thresholds following a 2 hour exposure to 115 dB SPL noise. Two weeks after exposure, thresholds returned to their preexposure level. In a study evaluating the possible interaction of sedation and NIHL in guinea pigs, Hildesheimer et al. reported temporary threshold shift of the action potential following exposure to a 20 minute, 4 kHz pure tone at 120 dB SPL.

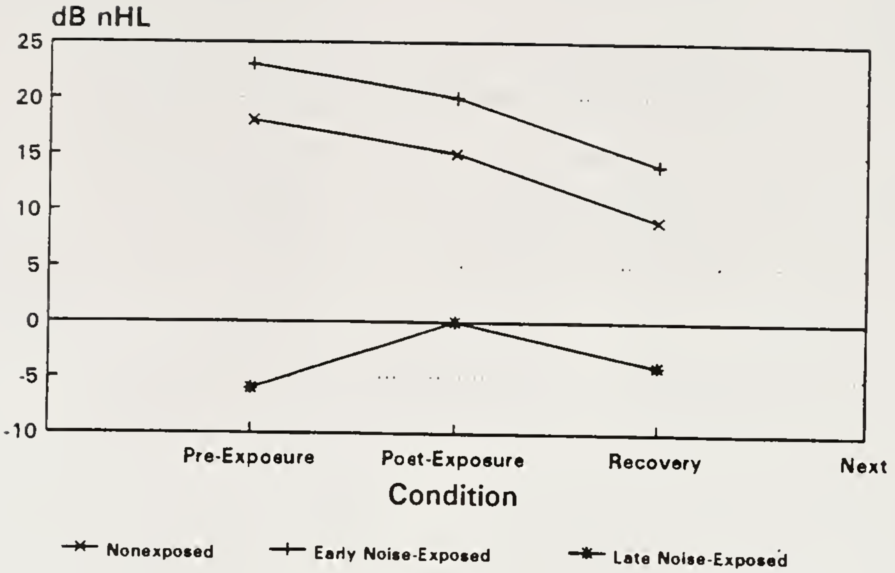
The lack of significance for the threshold changes evoked by the tone bursts may have been related to the area of excitation along the basilar membrane. Recall that low

Figure 3-13. Mean pre- and postexposure and recovery thresholds for the nonexposed, early noise-exposed (113 dGA) and late noise-exposed (130 dGA) groups. A. Click stimulus. B. 2 kHz stimulus. C. 1 kHz stimulus. D. 0.5 kHz stimulus.


dB nHL
20
10
0
-10
*
Pre-Exposure
Post-Exposure
Recovery
Next
Condition
Nonexposed
Early Noise-Exposed
Late Noise-Exposed

* p = 0.0062


dB nHL
30
20
10
0
-10
Pre-Exposure
Post-Exposure
Recovery
Next
Condition
Nonexposed
Early Noise-Exposed
Late Noise-Exposed



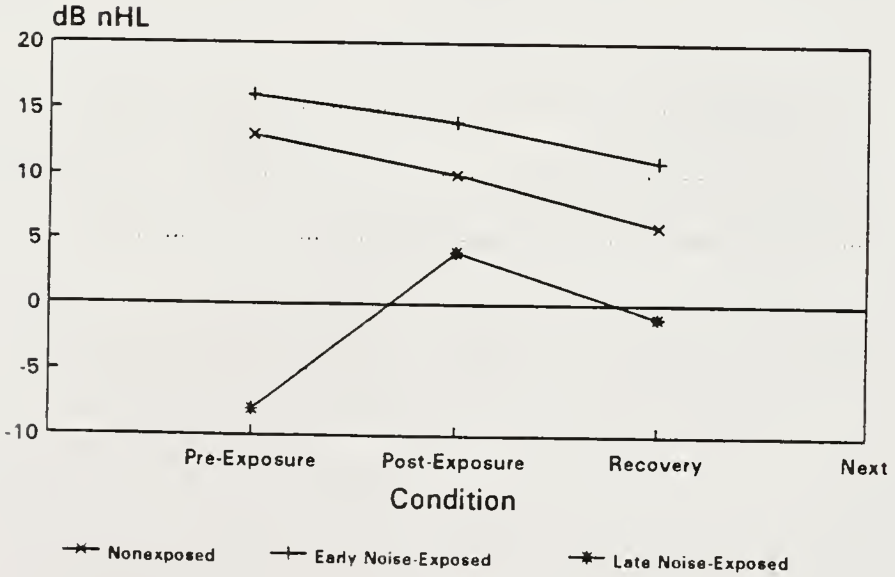


Figure 3-13. Continued

frequency ABR thresholds in the more developed fetus were not as sensitive as clicks or the higher frequency stimuli, because of the increased low frequency travel time along the basilar membrane (Kiang and Moxon, 1974; Davis and Hirsh, 1979). An alternative view could be that the noise effect was biased toward the high frequency basal end of the cochlea.

Thresholds for the early noise-exposed group did not increase after the exposure for any stimulus type. In fact, thresholds immediately after noise exposure decreased at nearly the same rate as for the nonexposed group. The lack of a threshold change for the early noise-exposed group did not seem unusual because of the poor preexposure thresholds. Poor sensitivity for all stimulus types would have made threshold changes difficult to detect. The improved sensitivity of the late noise-exposed group made changes in thresholds more likely to occur and easier to detect.

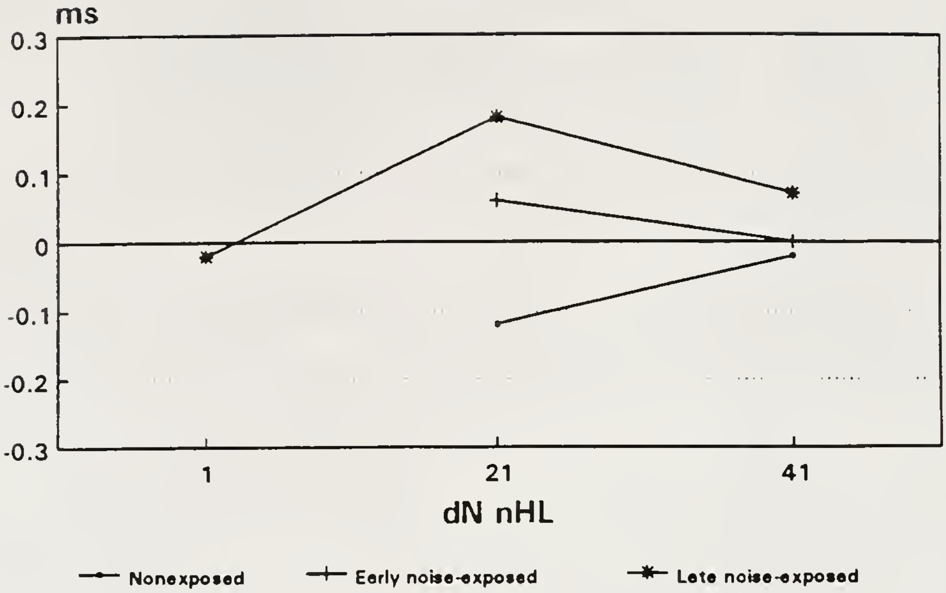
The separation between the mean nonexposed thresholds and the mean early noise-exposed thresholds at 1 kHz and 0.5 kHz was unusual and may be a reflection of the difficulty in recording ABR responses to low frequency stimuli. For moderate to high intensity levels, there is a spread of energy along the basilar membrane causing a low frequency stimulus to excite a higher frequency area (Gorga et al., 1988; Klein and Teas, 1978; Kiang and Moxon, 1974). This spread of energy could have resulted in greater variation among animals for the 1 kHz and 0.5 kHz tone burst stimuli. One would expect the mean preexposure thresholds for each of these two stimulus types to have been similar. The standard deviations for the 0.5 kHz and 1 kHz tone burst thresholds are somewhat greater than those for the click and 2 kHz thresholds. Refer to Appendix, Section A for standard deviations.

Latencies

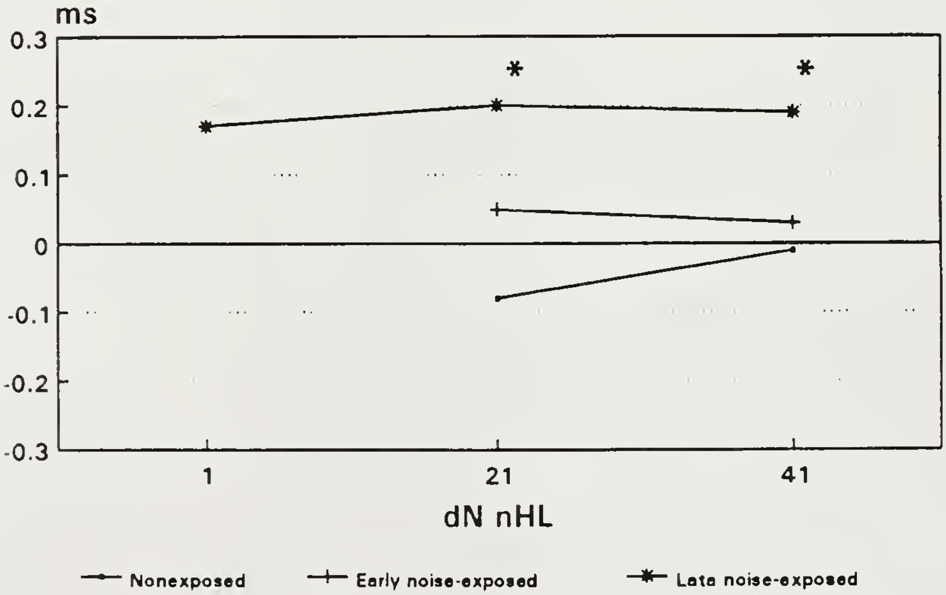
Appendix Section B contains the means, standard deviations, minimum and maximum latencies for Waves I-IV, the IV N trough, and amplitude for nonexposed, early and late noise-exposed animals. Recall that latency measurements were not available in the nonexposed and early-exposed groups until 116 dGA. Consequently, for the early noise-exposed group, there are no pre- and postexposure latencies to consider between 113 and 114 dGAs.

Figure 3-14 illustrates for all groups the click-evoked latency differences for dGA 131 minus dGA 130. For the late-exposed group, these differences represent pre- and post-noise exposure measurements. If the ABR recordings from these two days had been the same, the expected latency difference would have been 0 ms. When compared to the late noise-exposed group, all waves of the nonexposed and early noise-exposed groups demonstrated differences that were much closer to 0 ms. Analysis of variance for the three groups

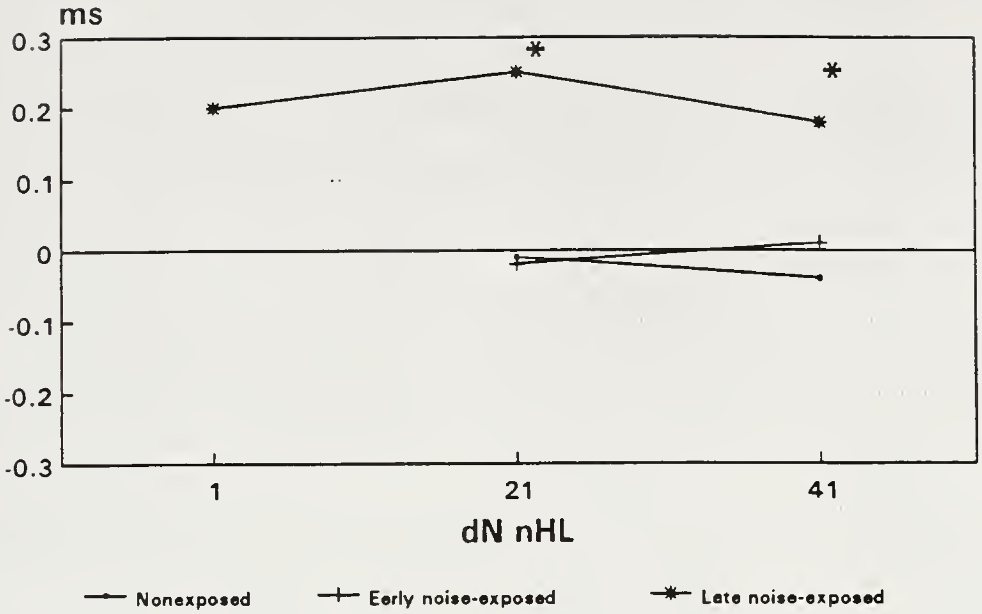
Figure 3-14. Mean click-evoked latency differences for dGA 131 minus dGA 130 for the nonexposed, early and late noise-exposed animals. Significant differences ($p < .05$) for Waves II, III, and IV at 41 and 21 dB nHL among the three groups are designated by asterisks.



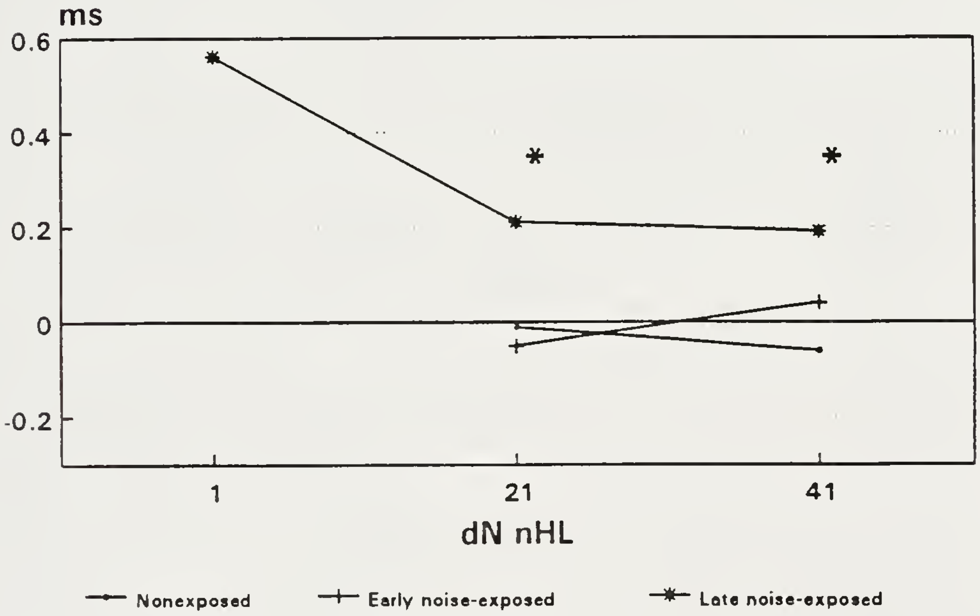
Wave I



Wave II (* $p < 0.05$)



Wave III (* p < 0.05)



Wave IV (* p < 0.05)

Figure 3-14. Continued

by stimulus level revealed that Waves II, III, and IV were significantly different ($p < 0.05$) for both 21 and 41 dB nHL.

Griffiths et al. (1989) cautioned that there is statistical dependence among succeeding ABR peaks. For example, Wave I is represented as a part of Wave II, then Wave III, and so on. This dependence was indeed reflected in the decreasing attained p-values when the latency differences among the three groups were compared. Table 3-1 illustrates the attained p-values for each wave for the click-evoked latency differences between dGA 130 and 131. With the exception of Wave IV, 60 dB, attained p-values decreased from Wave I to IV, thus indicating the dependence suggested by Griffiths et al. (1989). Consequently, no definite conclusion about brainstem loci of latency change is possible. However, the data did indicate that the noise exposure delivered to fetuses at 130 dGA prolonged the latencies of waveform peaks.

The ABR latency recorded from adults has frequently been used to gauge temporary and permanent noise-induced fluctuations (Almadori et al., 1988; Attias and Pratt, 1985, 1986; Attias et al., 1990; Sohmer et al., 1991). In a retrospective study in humans with presumed NIHL, Almadori et al. (1988) found latency values for Waves I through V to be within normal range. Attias et al. (1990) reported no latency changes for Wave I or for the interpeak I-V latency

Table 3-1. Attained p-values for click-evoked latency differences.

Level (dB nHL)	Wave I	Wave II	Wave III	Wave IV
41	0.3529	0.0044	0.0014	0.0006
21	0.0622	0.0047	0.0016	0.0298

in noise-exposed rats (115 dB SPL for 2 hours), even in the presence of a 24 dB threshold shift.

Conversely, Attias and Pratt (1985, 1986) reported latency changes that correlated with eventual hearing loss. In this human study, a click-evoked ABR was recorded after an exposure to 95 dB Hearing Level (HL) pink noise for 15 minutes. Recording from rats, Sohmer et al. (1991) found small latency changes in Waves I to IV following a 2-hour 115 dB SPL white-noise exposure.

The results of the current study agree with the work of Cook et al. (1981). Following noise exposure to the immature ear (115 dBA for 7.5 hours per day during pregnancy), these investigators found Wave IV latency of the guinea pig pup ABR to be prolonged. Latency increases corresponded to a 5 dB increase in stimulus level.

Cochlear Comparison

Nonexposed Subjects

Recall that four animals were sacrificed for histology only, two each at 119 dGA and 136 dGA. At 119 dGA, the cochleae appeared fully mature in all regions, i.e. the tunnel of Corti was identified in all turns. According to Pujol et al. (1991), the stage corresponding to the onset of cochlear function consists of an opening of the tunnel of Corti, opening of the Nuel spaces and the release of the tectorial membrane. These structures were apparent in all sections from all animals, indicating a time past the onset of function throughout all subjects. The identification of these structures was not surprising since ABRs were obtained from other animals prior to 119 dGA in the study.

In normal humans and experimental animals, once the onset of cochlear function had occurred, Pujol et al. (1991) reported only minor changes at the light microscope level. These changes included the elongation of the outer pillars and OHCs, and the development of Deiters' and Hensen's cells. Because of the plane of section artifacts, it was difficult to determine whether these changes had already occurred in the 119 dGA cochleae. Small amounts of mesenchyme, embryonic connective tissue, were found in the basal portions of scala tympani of all subjects at both GAs.

Figure 3-15A and 3-15B contain the cytocochleograms constructed from representative animals used only for histology at 119 and 136 dGAs, respectively. Depending on the orientation of the cochleae for cutting, artifacts (missing or damaged IHCs and OHCs) occurred either near the first turn or the upper turns.

Two animals were surgically prepared, but no ABR testing nor noise exposure were administered. The cochleae from these animals were prepared at 136 dGA. The cytocochleograms for these two animals can be found in Figure 3-15C, and reveal minor hair cell loss near the

Figure 3-15. Cytocochleograms from groups of subjects in this study. A. Histology only (119 dGA); B. Histology only (136 dGA); C. Surgery only; D. ABR only; E. Early noise-exposed (119 dGA); F. Early noise-exposed (136 dGA); G. Late noise-exposed (136 dGA).

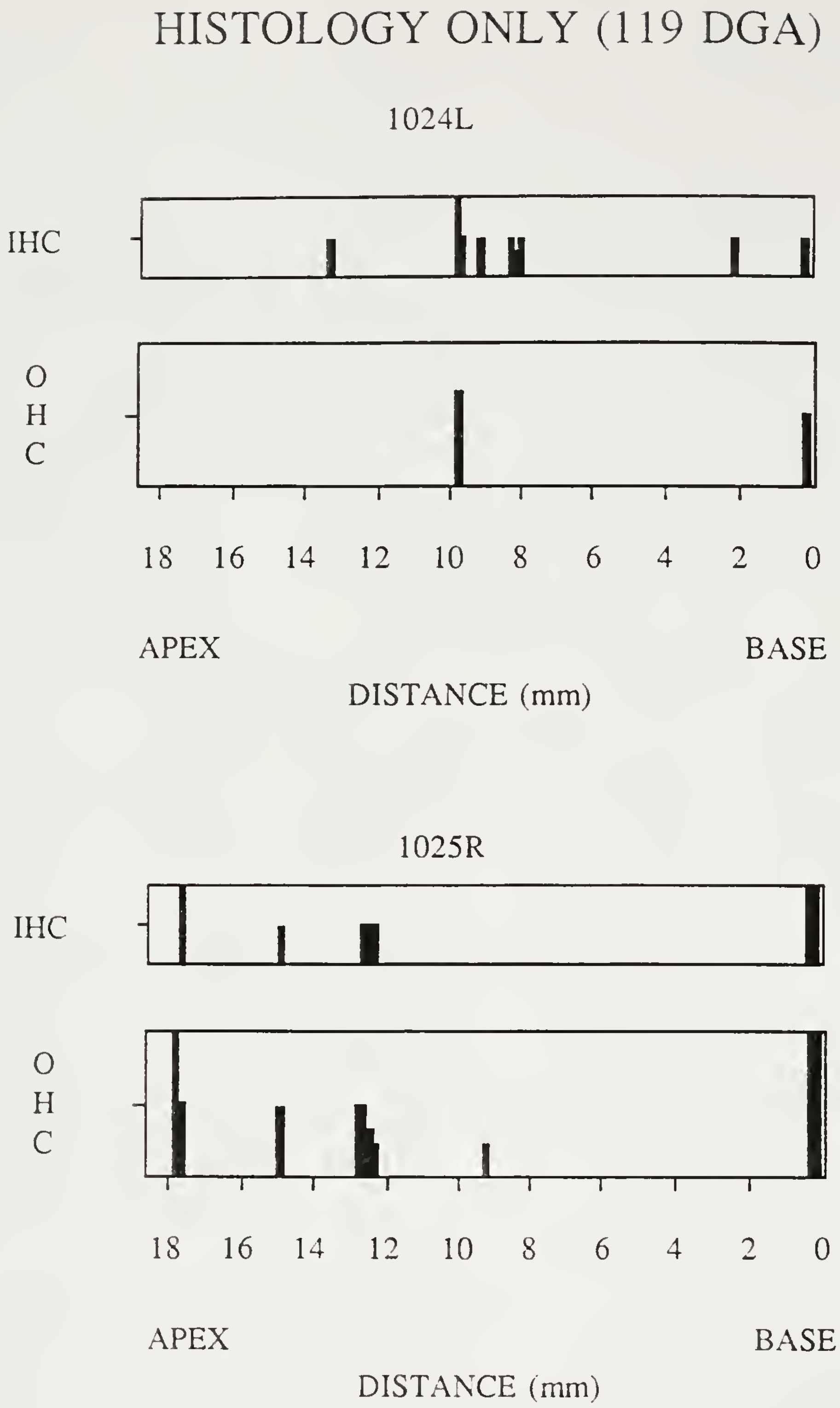


Figure 3-15. Continued.

HISTOLOGY ONLY (136 DGA)

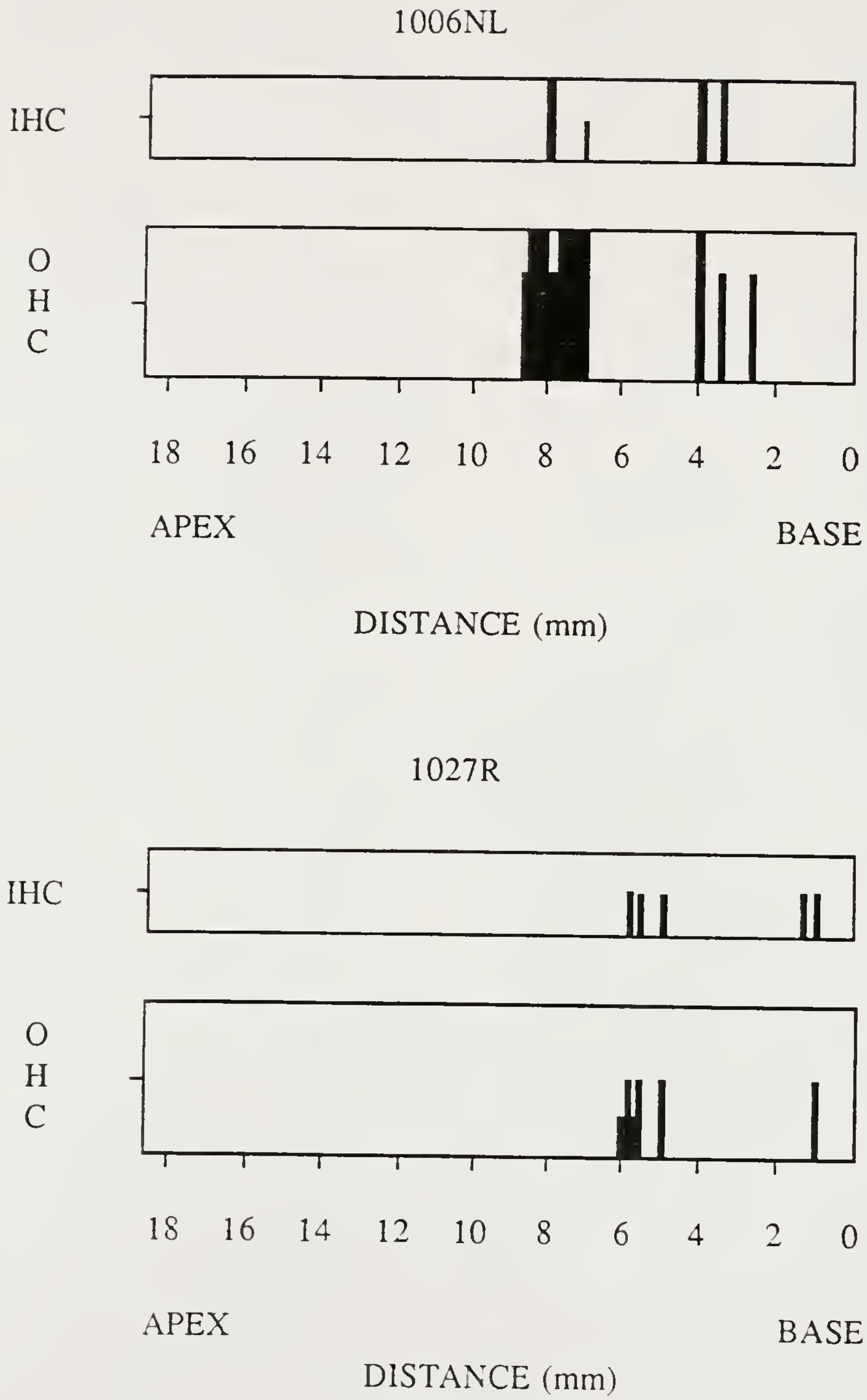


Figure 3-15. Continued.

SURGERY ONLY

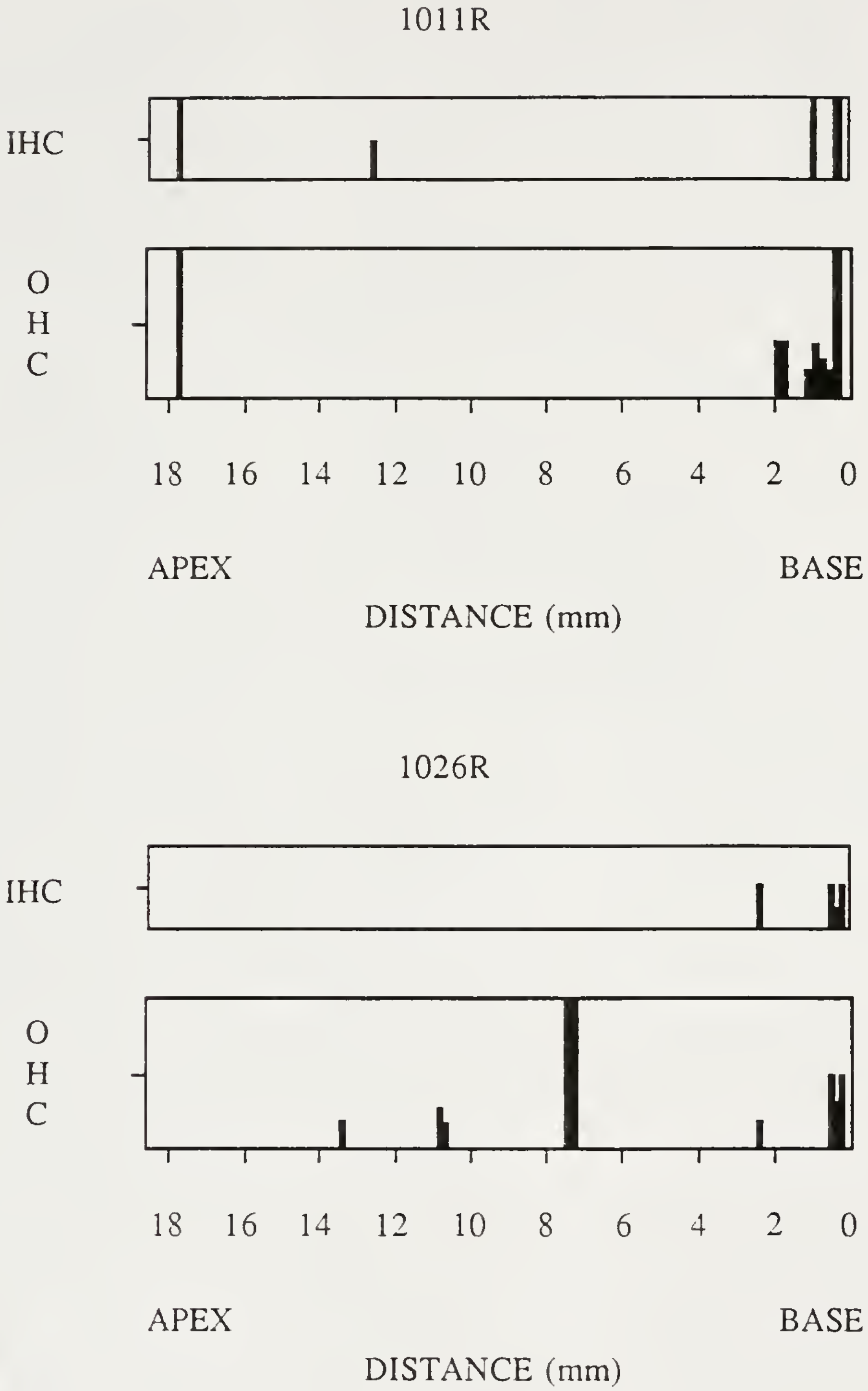


Figure 3-15. Continued.

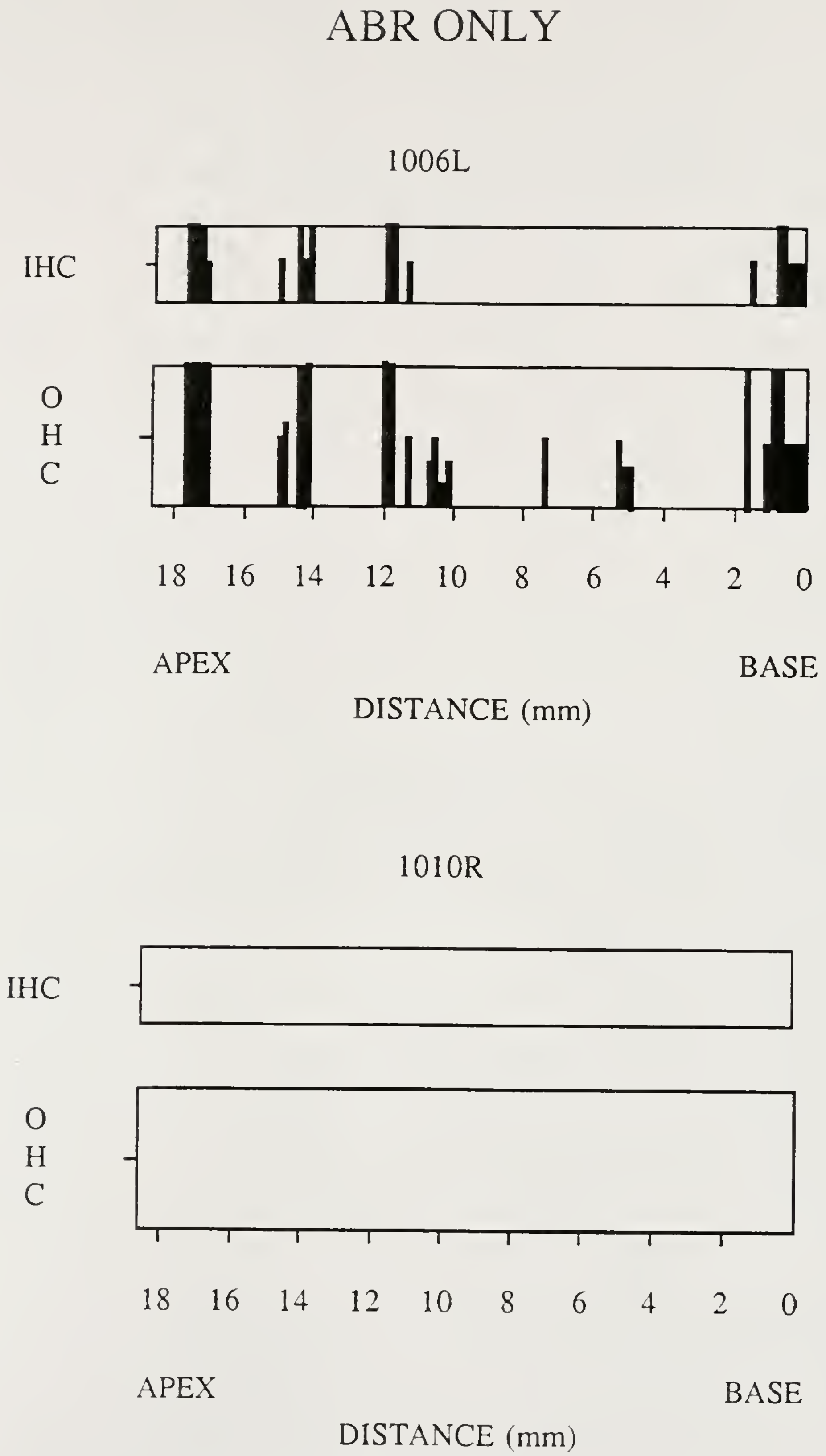


Figure 3-15. Continued.

EARLY NOISE-EXPOSED (119 DGA)

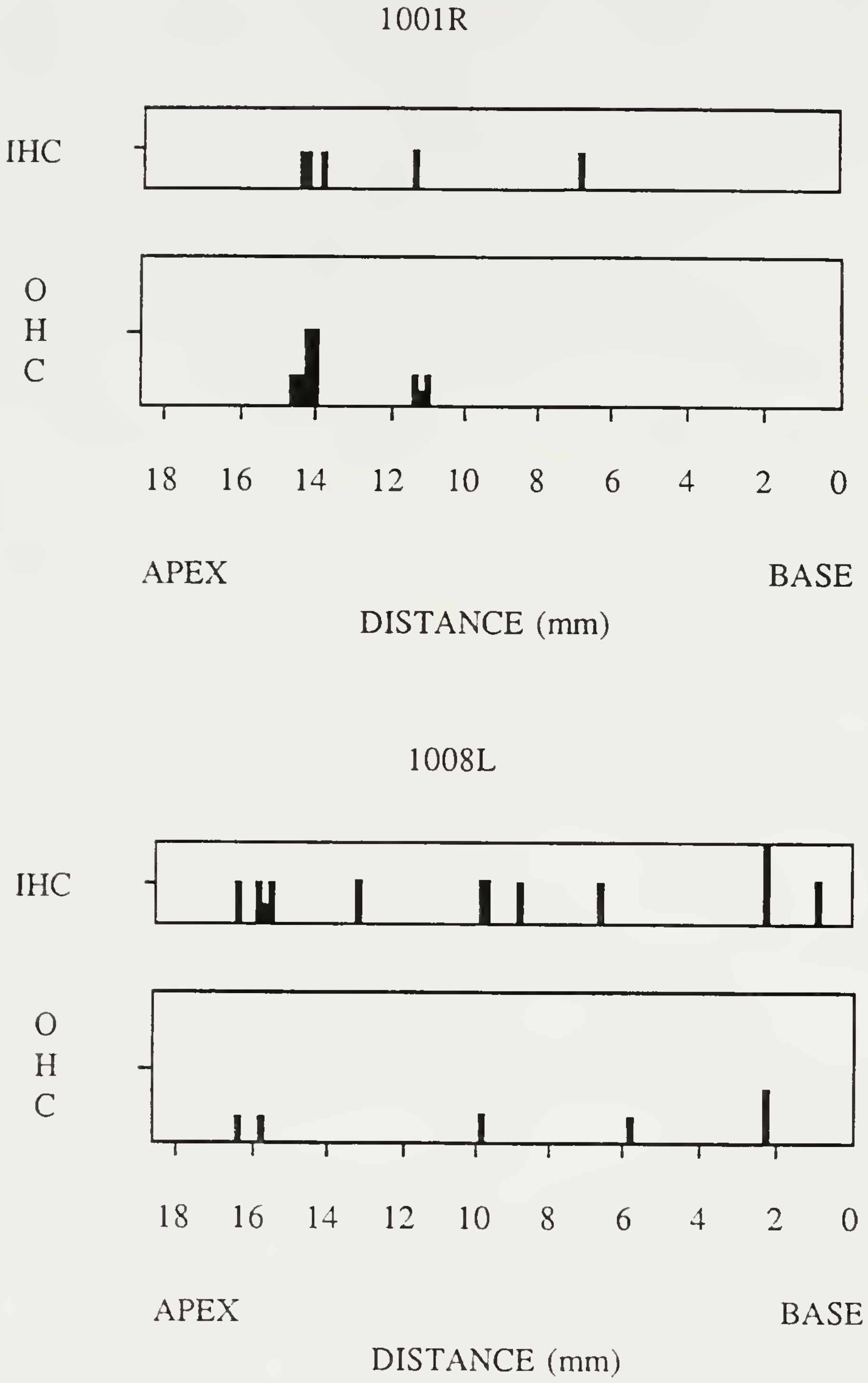


Figure 3-15. Continued.

EARLY NOISE-EXPOSED (136 DGA)

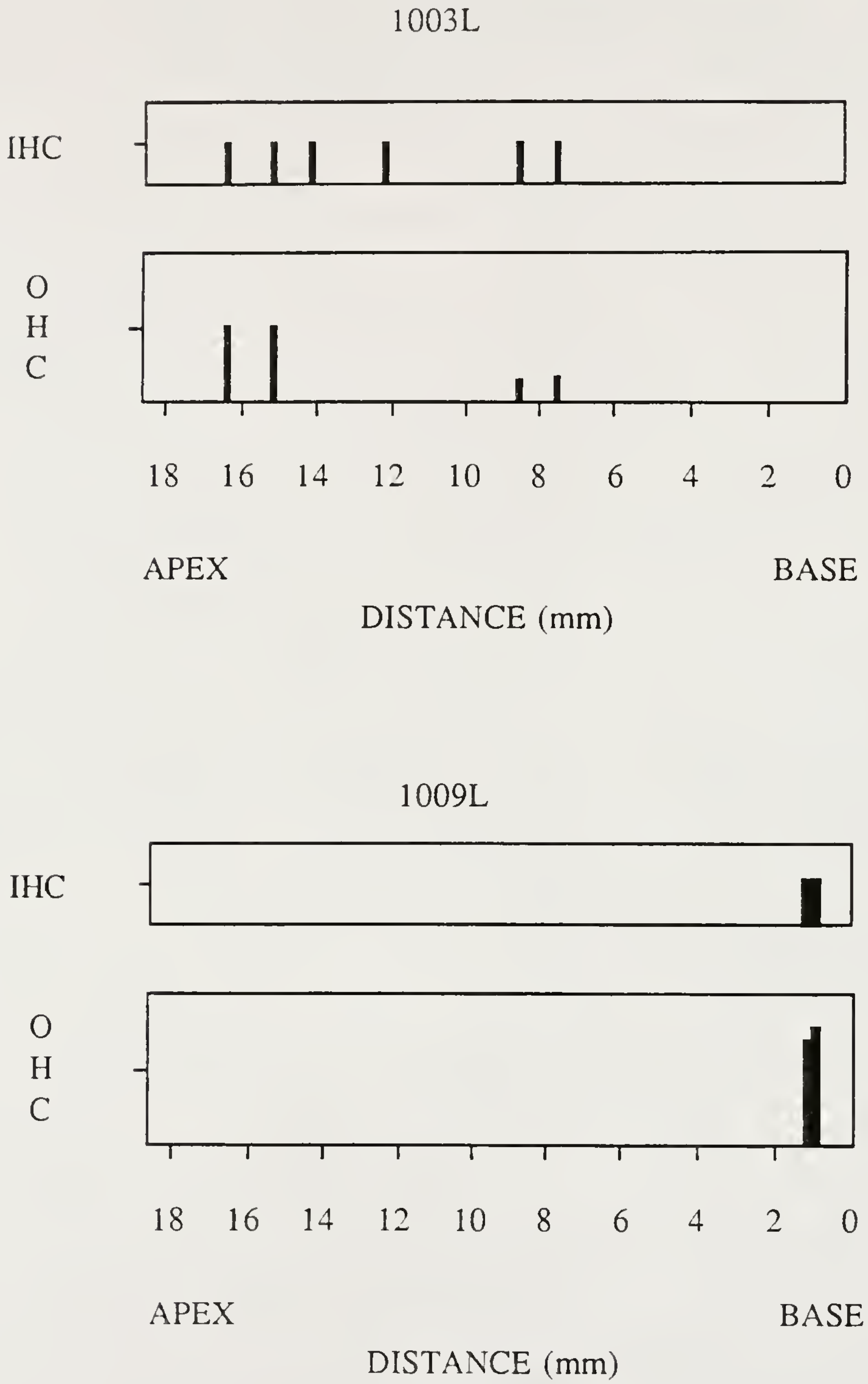


Figure 3-15. Continued.

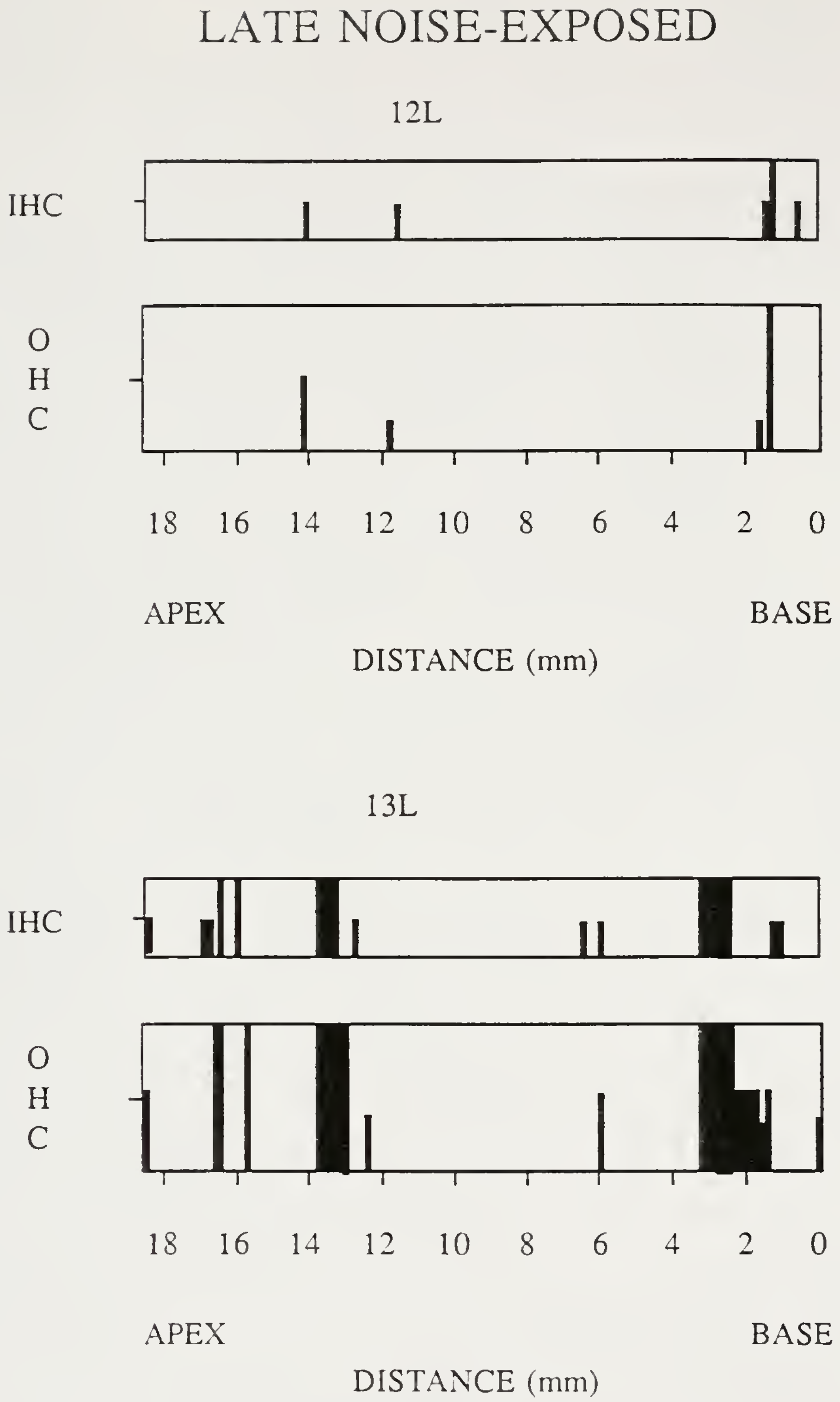


Figure 3-15. Continued

base for both animals and cell loss at the beginning of the first turn for Subject 1026R.

Animals in the nonexposed group were surgically prepared, tested with ABR, but did not participate in the noise exposure. Cochleae from two of these animals were removed at 136 dGA and their cytocochleograms can be found in Figure 3-15D. Subject 1006L showed a plane of section cutting artifact in the base and some of the upper turns. Reissner's membrane was broken throughout most of the cochlea. This cochlea was not completely decalcified, which almost certainly contributed to the amount of damage. Because the long basal turn did not appear in the same sections as the upper turns, estimates of basilar membrane length were used in Subject 1010. Otherwise, this cochlea had a normal appearance throughout.

Noise-Exposed Subjects

Cochleae from four of the early noise-exposed animals had been harvested at two GAs, two at 119 dGA and two at 136 dGA. The cytocochleograms from these four subjects appear in Figure 3-15E and 3-15F. Again because of cochlear orientation during cutting, the length of the cochleae from Subjects 1001R and 1008L could only be estimated. The IHCs and OHCs revealed scattered damage either near the base or towards the upper turns, depending on the plane of sectioning.

Cytocochleograms from two of the late noise-exposed animals are in Figure 3-15G. Subject 12L's cytocochleogram, with the exception of plane of section artifact in the base and upper turns, showed little damage. The cytocochleogram from Subject 13L consisted of far more damage in both the basal and upper turns. The cochleae from this subject had spiral ligament separation throughout nearly the entire length. This could have been artifact caused by an overly-long time of decalcification.

In the only other known study using serial celloidin section in sheep after noise exposure, Dunn et al. (1981) reported bulging Reissner's membranes, misshapen or indistinct supporting cells, collapsed or fused Reissner's or tectorial membrane in both their nonexposed and their fetally noise-exposed animals. Many of their observations were probably related to the problems of obtaining an appropriate plane of sectioning throughout the sheep cochlea. It seems that damaged areas viewed with light microscopy in the cytocochleograms from the current study are related to either under or over-decalcification or to plane of section problems that appeared to be unavoidable in the sheep cochlea. This finding is consistent with the Lenoir and Pujol (1980) study that found 7 days after the noise exposure, damage to young rat sensory and neural structures was apparent that could not be identified with light microscopy. In their study, with transmission electron microscopy, they found distortion of the majority of OHCs, while IHCs had less numerous changes.

CHAPTER IV
SUMMARY AND CONCLUSIONS

Three groups of fetal sheep were chronically instrumented for longitudinal ABR testing. *In utero* recordings were collected from 111 days gestational age to 136 days gestational age. The auditory brainstem response was evoked by clicks and tone bursts of 0.5 kHz, 1 kHz and 2 kHz delivered through a bone oscillator fastened to the fetal skull. Pregnant ewes were exposed to a broadband noise of 120 dB SPL for 16 hours at a gestational age of either 113 days or 130 days. A control group of animals was not exposed to noise. Selected animals were sacrificed, their cochleae removed, embedded in celloidin, cut and stained, and mounted on slides for light microscopy.

Nonexposed ABR and Cytocochleogram

The ABR showed considerable development of its morphology during the gestational period selected for this study. When the fetuses were first tested at 111 dGA, the morphology of the waveform was poorly defined. It was characterized by a rounded positive portion, occurring between 1 and 2 ms and a negative drop in amplitude, occurring after 6 to 7 ms. Peak identification could not typically occur until about 116 dGA. Morphology of the waveform continued to improve and was well-developed by 136 dGA in all animals. At this time, there were the four identifiable vertex-positive peaks typical of the sheep ABR. As the morphology of the ABR waveform improved, thresholds rapidly decreased, especially between 111 and 123 dGA. During this time, ABR sensitivity improved 3.17 dB/day. From 123 to 136 dGA, sensitivity improved at a slower rate, approximately 0.15 dB/day.

Although thresholds for the low frequency stimuli improved initially, thresholds for the higher frequency stimuli (click and 2 kHz), were lower than the 0.5 kHz threshold by the end of the study. These results are consistent with the development of the place principle (Rubel and Ryals, 1983). The ABR latencies for all four peaks also decreased during development from 111 to 136 dGA. These findings are consistent with improvements expected with increased myelination, axon diameter and the resultant improved neural synchrony. Latencies for Waves III and IV showed a greater decrease than latencies for Waves I and II. This finding could be related to differential rates of maturation of the peripheral and central auditory pathways, development of place specificity in the cochlea, or the dependent nature of the ABR peaks on the preceding peak.

Compared to humans and to other species typically used in hearing research, the sheep cochlea has an unusual shape. The organ of Corti consists of 2 turns, and is characterized by a very long basal turn of about 9 mm compared to the overall length of approximately 18 mm. The axis of the modiolus changes abruptly between the base and the middle turn. This change in axis was characterized by a 90 degree rotation in the plane of the organ of Corti.

Cochlear reconstructions of the serial celloidin sections were atypical, compared to other species, in that they had a "tear-drop" shape. Because of the bend in the modiolus of the fetal sheep cochlea, it was impossible to complete a preparation without artifact caused by rotation of the plane in some sections. Consequently, the cytocochleograms of nearly all animals showed some IHC and OHC damage, either in the basal turn or in the upper turns, depending on the plane of section.

Noise-Exposed ABR and Cytocochleogram

Although there was no immediate effect on the ABR of the animals exposed at the earlier gestational age (114 dGA), when these animals were tested again at a later gestational age (130 dGA), thresholds were higher than for animals that were not exposed to noise. These results indicated that while there was no immediate threshold shift after the noise exposure on 114 dGA, the ABRs for the early noise-exposed animals recorded 2 weeks or more subsequent to the noise exhibited higher thresholds than nonexposed animals. These findings contradicted the Dunn et al. (1981)

fetal sheep study, in which they reported no ABR threshold differences between a group of control animals and a group of lambs who had been prenatally exposed to noise.

Significant threshold and latency increases were identified immediately after the noise exposure for the group of animals exposed at 130 days gestational age. Latency-intensity functions for three of the four vertex-positive waves identified in the fetal sheep were prolonged after the noise. For all stimulus types, there was a trend of an increase in threshold immediately following the noise exposure (threshold shift) followed a decrease in threshold (recovery). The click-evoked threshold increase (7 dB) was significant. These findings are consistent with other NIHL studies, using ABR in other species, and indicate a small ABR threshold shift to click stimuli after noise exposure (Attias et al., 1990; Hildesheimer et al. 1991).

With the exception of one cochlea that demonstrated preparation artifact, there were no differences between the nonexposed and noise-exposed cytocochleograms at the light microscopy level. However, Lenoir and Pujol (1980) found that 7 days after a noise exposure, damage to sensory and neural structures was apparent under electron microscopy that could not be identified with light microscopy.

Previous studies have shown that exogenous sounds penetrate the uterus with little reduction in sound pressure for frequencies less than 0.25 kHz (Gerhardt et al., 1990).

In fact, a 5 dB enhancement of some low frequency sounds has been reported in sheep and in humans (Gerhardt et al., 1990; Richards et al., 1992). The capability of the hearing mechanism to respond to these exogenous sounds and the possibility of damage to the hearing mechanism from these sounds have been unknown.

The present study confirms that sounds penetrate the uterus and stimulate fetal hearing. The intense exposures used in this study (probably never experienced in any work environment) can cause ABR changes in fetal sheep. The extension of the findings in this study to humans necessitate an understanding of the effects of noise exposure on the adult sheep ABR. These data are currently not available. However, it is important to note the findings of Lalande et al. (1986) and Daniel and Laciak (1982). They found an increased risk of hearing loss in children whose mothers were occupationally exposed to hazardous noise. These studies have been criticized (Henderson et al., 1993) for methodological shortcomings, such a lack of a matched control group.

Results from this study showed that exogenous intense noise can penetrate the uterus, stimulate the fetal auditory system and create temporary shifts in the ABR during late gestational exposures and perhaps temporary or permanent changes with earlier gestational exposures that are revealed as a retardation of development 2 weeks later. Complicated by the unusual shape of the fetal sheep cochlea, no differences between nonexposed and noise-exposed animals appeared at under light microscopy.

Implications for Research

An interesting area of study in hearing research is the identification of a "critical period" for increased damage following noise exposure in humans. Critical periods of susceptibility to noise exposure of young animals have been suggested in many species. From looking at the organ of Corti, Falk et al. (1974) determined that newborn guinea pigs were more susceptible to noise-induced hearing loss (NIHL) than adult guinea pigs. Price (1976) had a similar finding comparing the CM in kittens to mature cats. Bock and Saunders (1977) identified a critical period for noise-induced hearing loss in young hamsters that occurred after onset of the CM and in what appeared to be maturation of the cochlea as assessed with light microscopy. From recordings of the action potential, Lenoir et al. (1979) described a period in the rat pup when a permanent NIHL was produced at a level that would only cause temporary changed in the adult rat.

It is now known that intense noise exposure to fetal sheep at two different GAs produces changes in the fetal ABR. The possibility of an *in utero* critical period could be investigated by comparing the noise exposure to young lambs or adult ewes. Furthermore, the effects of different spectra on the developing fetus could be investigated with particular emphasis given to the period between 111 and 116 dGA. This appears to be a period when physiological and structural changes occur resulting in rapid ABR development.

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APPENDIX

The following tables represent output from SAS™ of mean click and tone-burst ABR evoked thresholds (A) and latencies (B) for nonexposed, early noise-exposed and late noise-exposed subjects.

Group Codes:

0 = Nonexposed
1 = Early Noise-Exposed
2 = Late Noise-Exposed

A. THRESHOLDS

Click

Nonexposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	9	67.7777778	9.0523171	55.0000000	80.0000000
DAY 113	9	60.0000000	7.5000000	50.0000000	70.0000000
DAY 114	9	58.3333333	9.0138782	45.0000000	70.0000000
DAY 116	9	53.8888889	6.5085414	45.0000000	65.0000000
DAY 123	7	37.1428571	6.3620901	30.0000000	45.0000000
DAY 130	6	33.3333333	6.8313005	25.0000000	45.0000000
DAY 131	6	30.0000000	3.1622777	25.0000000	35.0000000
DAY 136	5	28.0000000	5.7008771	20.0000000	35.0000000

Early Noise-Exposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	13	67.3076923	6.6506217	60.0000000	80.0000000
DAY 113	12	60.0000000	6.3960215	50.0000000	70.0000000
DAY 114	12	58.3333333	6.1545745	45.0000000	65.0000000
DAY 116	10	51.5000000	5.7975090	45.0000000	60.0000000
DAY 123	7	35.7142857	5.3452248	30.0000000	45.0000000
DAY 130	6	35.8333333	7.3598007	25.0000000	45.0000000
DAY 131	5	36.0000000	7.4161985	25.0000000	45.0000000
DAY 136	5	35.0000000	10.0000000	25.0000000	50.0000000

Late Noise-Exposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	0	.	.	.	.
DAY 113	0	.	.	.	.
DAY 114	0	.	.	.	.
DAY 116	0	.	.	.	.
DAY 123	0	.	.	.	.
DAY 130	6	25.0000000	7.7459667	10.0000000	30.0000000
DAY 131	6	35.8333333	6.6458007	30.0000000	45.0000000
DAY 136	6	28.3333333	4.0824829	25.0000000	35.0000000

2 kHz

Nonexposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	9	71.6666667	8.6602540	55.0000000	85.0000000
DAY 113	9	64.4444444	5.8333333	60.0000000	75.0000000
DAY 114	9	57.7777778	9.7182532	45.0000000	75.0000000
DAY 116	9	51.1111111	7.4068287	40.0000000	65.0000000
DAY 123	7	34.2857143	10.1770049	20.0000000	50.0000000
DAY 130	6	28.3333333	5.1639778	20.0000000	35.0000000
DAY 131	3	26.6666667	10.4083300	15.0000000	35.0000000
DAY 136	5	32.0000000	8.3666003	20.0000000	40.0000000

Early Noise-Exposed

```
Variable   N          Mean        Std Dev        Minimum        Maximum
-------------------------------------------------------------------------
DAY 111   13    71.1538462      5.8287528     65.0000000     85.0000000
DAY 113   12    64.5833333      8.3824311     50.0000000     80.0000000
DAY 114   12    60.8333333      9.7312368     45.0000000     75.0000000
DAY 116   10    52.0000000      8.8819417     40.0000000     65.0000000
DAY 123    7    39.2857143      8.8640526     30.0000000     55.0000000
DAY 130    6    35.8333333      6.6458007     25.0000000     45.0000000
DAY 131    4    37.5000000      6.4549722     30.0000000     45.0000000
DAY 136    5    39.0000000     14.7478812     30.0000000     65.0000000
-------------------------------------------------------------------------
```

Late Noise-Exposed

```
Variable   N          Mean        Std Dev        Minimum        Maximum
-------------------------------------------------------------------------
DAY 111    0             .              .              .              .
DAY 113    0             .              .              .              .
DAY 114    0             .              .              .              .
DAY 116    0             .              .              .              .
DAY 123    0             .              .              .              .
DAY 130    6    25.0000000      7.7459667     15.0000000     35.0000000
DAY 131    6    31.6666667      8.7559504     20.0000000     45.0000000
DAY 136    5    26.0000000      4.1833001     20.0000000     30.0000000
-------------------------------------------------------------------------
```

1 kHz

Nonexposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	9	60.5555556	8.8191710	50.0000000	75.0000000
DAY 113	9	51.6666667	6.1237244	45.0000000	60.0000000
DAY 114	9	49.4444444	9.8248551	30.0000000	60.0000000
DAY 116	9	42.7777778	9.0523171	25.0000000	60.0000000
DAY 123	7	34.2857143	13.3630621	15.0000000	55.0000000
DAY 130	6	23.3333333	4.0824829	20.0000000	30.0000000
DAY 131	4	25.0000000	4.0824829	20.0000000	30.0000000
DAY 136	5	18.0000000	5.7008771	10.0000000	25.0000000

Early Noise-Exposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	13	63.4615385	11.4354375	45.0000000	85.0000000
DAY 113	12	56.6666667	8.8762536	40.0000000	70.0000000
DAY 114	12	53.7500000	9.3237234	45.0000000	75.0000000
DAY 116	10	48.0000000	6.7494856	35.0000000	55.0000000
DAY 123	7	29.2857143	6.7259271	20.0000000	40.0000000
DAY 130	6	25.8333333	7.3598007	15.0000000	35.0000000
DAY 131	4	25.0000000	7.0710678	15.0000000	30.0000000
DAY 136	5	28.0000000	15.6524758	5.0000000	45.0000000

Late Noise-Exposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	0	.	.	.	.
DAY 113	0	.	.	.	.
DAY 114	0	.	.	.	.
DAY 116	0	.	.	.	.
DAY 123	0	.	.	.	.
DAY 130	6	28.3333333	8.7559504	20.0000000	45.0000000
DAY 131	6	34.1666667	10.6848803	20.0000000	50.0000000
DAY 136	5	30.0000000	8.6602540	25.0000000	45.0000000

.5 kHz

Nonexposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	9	70.0000000	8.6602540	60.0000000	85.0000000
DAY 113	9	59.4444444	8.4574096	45.0000000	75.0000000
DAY 114	9	55.5555556	10.1379376	40.0000000	75.0000000
DAY 116	9	51.6666667	7.9056942	40.0000000	65.0000000
DAY 123	7	49.2857143	6.7259271	40.0000000	55.0000000
DAY 130	6	42.5000000	6.8920244	35.0000000	55.0000000
DAY 131	5	46.0000000	12.4498996	35.0000000	65.0000000
DAY 136	5	44.0000000	14.7478812	30.0000000	65.0000000

Early Noise-Exposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	13	68.0769231	9.2507796	55.0000000	85.0000000
DAY 113	12	62.0833333	8.3824311	45.0000000	75.0000000
DAY 114	12	60.4166667	8.9082019	45.0000000	75.0000000
DAY 116	10	57.0000000	5.8689390	45.0000000	65.0000000
DAY 123	7	52.1428571	4.8795004	45.0000000	55.0000000
DAY 130	6	49.1666667	7.3598007	40.0000000	55.0000000
DAY 131	4	56.2500000	6.2915287	50.0000000	65.0000000
DAY 136	5	55.0000000	10.6066017	40.0000000	65.0000000

Late Noise-Exposed

Variable	N	Mean	Std Dev	Minimum	Maximum
DAY 111	0	.	.	.	.
DAY 113	0	.	.	.	.
DAY 114	0	.	.	.	.
DAY 116	0	.	.	.	.
DAY 123	0	.	.	.	.
DAY 130	6	38.3333333	10.3279556	20.0000000	50.0000000
DAY 131	6	50.0000000	7.0710678	40.0000000	60.0000000
DAY 136	4	45.0000000	10.8012345	30.0000000	55.0000000

B. LATENCIES

Click

Nonexposed dGA=111 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	1.9000000	.	1.9000000	1.9000000
WAVE II	1	2.6600000	.	2.6600000	2.6600000
WAVE III	1	3.2800000	.	3.2800000	3.2800000
WAVE IV	1	3.8400000	.	3.8400000	3.8400000
TROUGH	0	.	.	.	.
AMPLITUDE	0	.	.	.	.

Nonexposed dGA=113 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	4	1.9625000	0.4830718	1.2900000	2.4400000
WAVE II	4	2.9400000	0.5520869	2.2200000	3.5400000
WAVE III	2	4.7350000	0.4030509	4.4500000	5.0200000
WAVE IV	3	5.2333333	0.4277071	4.7400000	5.5000000
TROUGH	2	6.6250000	0.2474874	6.4500000	6.8000000
AMPLITUDE	2	0.6500000	0.1131371	0.5700000	0.7300000

Nonexposed dGA=114 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	2.4100000	.	2.4100000	2.4100000
WAVE II	1	3.3800000	.	3.3800000	3.3800000
WAVE III	1	4.8300000	.	4.8300000	4.8300000
WAVE IV	2	5.3100000	0.8909545	4.6800000	5.9400000
TROUGH	2	6.5100000	0.1838478	6.3800000	6.6400000
AMPLITUDE	2	0.2950000	0.0353553	0.2700000	0.3200000

Nonexposed dGA=114 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	5	1.8860000	0.4376985	1.2200000	2.3900000
WAVE II	5	2.8000000	0.5805601	1.8400000	3.3500000
WAVE III	5	4.0540000	0.9163951	2.8600000	4.9800000
WAVE IV	4	5.0225000	0.4038461	4.6400000	5.5000000
TROUGH	4	6.4375000	0.5552402	5.6600000	6.8800000
AMPLITUDE	4	0.5975000	0.1252664	0.4700000	0.7100000

Nonexposed dGA=116 LEVEL=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	2	2.5400000	0.4242641	2.2400000	2.8400000
WAVE II	2	3.7500000	0.5515433	3.3600000	4.1400000
WAVE III	4	4.8325000	0.8311989	3.7000000	5.6800000
WAVE IV	4	6.2300000	0.3725587	5.9600000	6.7800000
TROUGH	4	7.1725000	0.4719728	6.7200000	7.8000000
AMPLITUDE	4	0.4375000	0.0865544	0.3400000	0.5400000

Nonexposed dGA=116 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	8	1.9200000	0.3438854	1.1500000	2.2200000
WAVE II	8	2.8925000	0.4997356	2.0800000	3.3700000
WAVE III	8	4.1600000	0.6766936	3.0800000	4.9200000
WAVE IV	8	5.3175000	0.6280525	4.4800000	6.2300000
TROUGH	8	6.9400000	0.4995140	6.3800000	7.6400000
AMPLITUDE	8	0.4900000	0.1631826	0.2200000	0.6900000

```
                   Nonexposed dGA=123 Level=40

Variable     N        Mean        Std Dev       Minimum       Maximum
----------------------------------------------------------------------
WAVE I       0           .              .             .             .
WAVE II      0           .              .             .             .
WAVE III     0           .              .             .             .
WAVE IV      2   6.5000000      0.4242641     6.2000000     6.8000000
TROUGH       2   7.6400000      0.2545584     7.4600000     7.8200000
AMPLITUDE    2   0.5050000      0.2192031     0.3500000     0.6600000
----------------------------------------------------------------------

                   Nonexposed dGA=123 Level=60

Variable     N        Mean        Std Dev       Minimum       Maximum
----------------------------------------------------------------------
WAVE I       5   2.5660000      0.2106656     2.4000000     2.8600000
WAVE II      6   3.4550000      0.3727600     2.9000000     3.9600000
WAVE III     7   4.7042857      0.2933063     4.2200000     5.1500000
WAVE IV      7   5.5785714      0.3335880     5.1800000     6.1600000
TROUGH       7   6.6385714      0.3492100     6.1800000     7.2500000
AMPLITUDE    7   0.7100000      0.2899425     0.3200000     1.0900000
----------------------------------------------------------------------

                   Nonexposed dGA=123 Level=80

Variable     N        Mean        Std Dev       Minimum       Maximum
----------------------------------------------------------------------
WAVE I       7   1.8814286      0.1322156     1.7400000     2.0500000
WAVE II      7   2.8571429      0.1512960     2.6500000     3.1200000
WAVE III     7   4.0657143      0.3169047     3.5100000     4.5400000
WAVE IV      7   4.9100000      0.3566978     4.4800000     5.4800000
TROUGH       7   6.0857143      0.3026471     5.7800000     6.4900000
AMPLITUDE    7   1.1514286      0.3476794     0.6800000     1.8000000
----------------------------------------------------------------------
```

Nonexposed dGA=130 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	4.3000000	.	4.3000000	4.3000000
WAVE III	1	5.0600000	.	5.0600000	5.0600000
WAVE IV	4	6.0050000	0.2734349	5.7800000	6.4000000
TROUGH	4	7.2800000	0.4329742	6.9000000	7.8800000
AMPLITUDE	4	0.6200000	0.2427619	0.4000000	0.8400000

Nonexposed dGA=130 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	5	2.6740000	0.1686120	2.5500000	2.9700000
WAVE II	6	3.4650000	0.2582828	3.2100000	3.9500000
WAVE III	6	4.5683333	0.1930199	4.3500000	4.9000000
WAVE IV	6	5.2916667	0.2758562	5.0200000	5.7000000
TROUGH	6	6.2400000	0.1635848	6.0200000	6.4500000
AMPLITUDE	6	0.9750000	0.2653865	0.5000000	1.2300000

Nonexposed dGA=130 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	6	1.8300000	0.0995992	1.7000000	1.9500000
WAVE II	6	2.7200000	0.1043072	2.5700000	2.8500000
WAVE III	6	4.0050000	0.1626960	3.7600000	4.2500000
WAVE IV	6	4.6933333	0.2812591	4.2600000	5.0300000
TROUGH	6	5.7100000	0.2481129	5.4300000	6.1200000
AMPLITUDE	6	1.2816667	0.4058284	0.7400000	1.7900000

```
                    Nonexposed dGA=131 Level=40

Variable    N        Mean         Std Dev         Minimum         Maximum
--------------------------------------------------------------------------
WAVE I      0           .               .               .               .
WAVE II     0           .               .               .               .
WAVE III    0           .               .               .               .
WAVE IV     2   6.1700000       0.3252691       5.9400000       6.4000000
TROUGH      2   7.2100000       0.1272792       7.1200000       7.3000000
AMPLITUDE   2   0.5350000       0.3464823       0.2900000       0.7800000
--------------------------------------------------------------------------

                    Nonexposed dGA=131 Level=60

Variable    N        Mean         Std Dev         Minimum         Maximum
--------------------------------------------------------------------------
WAVE I      6   2.5783333       0.1799352       2.2800000       2.7700000
WAVE II     6   3.3783333       0.2584892       3.0200000       3.7400000
WAVE III    6   4.5550000       0.1348703       4.4000000       4.7500000
WAVE IV     6   5.2866667       0.1963331       5.0700000       5.5700000
TROUGH      6   6.2233333       0.1445914       6.0000000       6.3900000
AMPLITUDE   6   1.2850000       0.9592862       0.6400000       3.1900000
--------------------------------------------------------------------------

                    Nonexposed dGA=131 Level=80

Variable    N        Mean         Std Dev         Minimum         Maximum
--------------------------------------------------------------------------
WAVE I      6   1.8100000       0.0923038       1.7400000       1.9900000
WAVE II     6   2.7066667       0.1157008       2.6100000       2.8800000
WAVE III    6   3.9700000       0.1608726       3.8200000       4.2600000
WAVE IV     6   4.6350000       0.2350106       4.2600000       4.8900000
TROUGH      6   5.6700000       0.2155922       5.4200000       6.0000000
AMPLITUDE   6   1.2733333       0.3484633       0.8200000       1.7400000
--------------------------------------------------------------------------
```

Nonexposed dGA=136 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	3.1800000	.	3.1800000	3.1800000
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	5	5.7780000	0.2777049	5.5000000	6.1600000
TROUGH	5	6.9420000	0.3268333	6.4800000	7.3200000
AMPLITUDE	5	0.6400000	0.2919760	0.3700000	1.0600000

Nonexposed dGA=136 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	5	2.5240000	0.2127910	2.2400000	2.8200000
WAVE II	5	3.3600000	0.2943637	3.1200000	3.8600000
WAVE III	5	4.4020000	0.1868689	4.1800000	4.6600000
WAVE IV	5	5.0820000	0.2051097	4.8700000	5.3500000
TROUGH	5	5.9740000	0.1346477	5.7500000	6.1000000
AMPLITUDE	5	1.0580000	0.2834960	0.6500000	1.3700000

Nonexposed dGA=136 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	5	1.7520000	0.0739594	1.6800000	1.8500000
WAVE II	5	2.6080000	0.1347961	2.5000000	2.7700000
WAVE III	5	3.8520000	0.1666433	3.6800000	4.0600000
WAVE IV	5	4.5880000	0.1321741	4.4400000	4.7300000
TROUGH	5	5.4320000	0.1834939	5.2300000	5.7000000
AMPLITUDE	5	1.3860000	0.4589989	0.9100000	2.0500000

Early Noise-Exposed dGA=111 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	4	1.2225000	0.0250000	1.1900000	1.2500000
WAVE II	4	2.1850000	0.0888819	2.1100000	2.3000000
WAVE III	4	3.2500000	0.3221801	2.9300000	3.6400000
WAVE IV	2	3.8850000	0.0636396	3.8400000	3.9300000
TROUGH	2	6.0800000	1.8667619	4.7600000	7.4000000
AMPLITUDE	2	0.6800000	0.5515433	0.2900000	1.0700000

Early Noise-Exposed dGA=113 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	2.6600000	.	2.6600000	2.6600000
WAVE II	4	3.2750000	0.5041494	2.7600000	3.7700000
WAVE III	3	4.2766667	0.7922331	3.7000000	5.1800000
WAVE IV	2	5.3000000	1.2727922	4.4000000	6.2000000
TROUGH	2	6.7900000	1.6546299	5.6200000	7.9600000
AMPLITUDE	2	0.3850000	0.0494975	0.3500000	0.4200000

Early Noise-Exposed dGA=113 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	8	1.3950000	0.3270649	1.1000000	1.9700000
WAVE II	8	2.3425000	0.4114348	2.0600000	3.1400000
WAVE III	8	3.5862500	0.6343487	2.9200000	4.6500000
WAVE IV	7	4.7157143	0.6993296	3.6100000	5.7500000
TROUGH	7	7.0914286	0.3964185	6.4700000	7.4200000
AMPLITUDE	7	0.5757143	0.2955705	0.2000000	1.0800000

Early Noise-Exposed dGA=114 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	2.5300000	.	2.5300000	2.5300000
WAVE II	3	3.4866667	0.5050083	2.9100000	3.8500000
WAVE III	2	4.6550000	0.9121677	4.0100000	5.3000000
WAVE IV	3	6.1600000	0.1509967	6.0200000	6.3200000
TROUGH	3	7.3266667	0.3629509	6.9400000	7.6600000
AMPLITUDE	3	0.3333333	0.0550757	0.2800000	0.3900000

Early Noise-Exposed dGA=114 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	9	1.4277778	0.3443028	1.0100000	1.9800000
WAVE II	9	2.6900000	0.5392124	1.8300000	3.2300000
WAVE III	9	3.9744444	0.7022840	2.8800000	4.7900000
WAVE IV	9	5.1255556	0.6302998	3.6400000	5.8100000
TROUGH	9	6.7022222	0.6572819	5.4000000	7.7300000
AMPLITUDE	9	0.4866667	0.2403123	0.1200000	0.9800000

Early Noise-Exposed dGA=116 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.7866667	0.2419366	2.6000000	3.0600000
WAVE II	5	3.0500000	0.6296030	2.3400000	3.9500000
WAVE III	6	4.4300000	0.8215108	3.5700000	5.3200000
WAVE IV	6	5.6866667	0.4074146	5.0800000	6.2100000
TROUGH	6	7.1833333	0.2534298	6.6800000	7.3600000
AMPLITUDE	6	0.4733333	0.1950043	0.2700000	0.7700000

Early Noise-Exposed dGA=116 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	8	1.4925000	0.3557587	1.1800000	1.9500000
WAVE II	9	2.4788889	0.4953899	1.9700000	3.0800000
WAVE III	9	3.7311111	0.6802471	2.9700000	4.6200000
WAVE IV	9	4.9288889	0.5317529	3.8800000	5.7700000
TROUGH	9	6.6744444	0.4299451	5.7400000	7.2900000
AMPLITUDE	9	0.6600000	0.2672546	0.2300000	1.0200000

Early Noise-Exposed dGA=123 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	2	6.6300000	0.1272792	6.5400000	6.7200000
TROUGH	2	7.5000000	0.1131371	7.4200000	7.5800000
AMPLITUDE	2	0.2950000	0.0494975	0.2600000	0.3300000

Early Noise-Exposed dGA=123 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.6466667	0.3010537	2.3100000	2.8900000
WAVE II	5	2.9920000	0.5473299	2.4100000	3.6200000
WAVE III	7	4.2642857	0.7836848	3.2800000	5.1700000
WAVE IV	7	5.3814286	0.5379724	4.5600000	6.0200000
TROUGH	7	6.6571429	0.2689928	6.2600000	6.9200000
AMPLITUDE	7	0.8271429	0.3357933	0.4600000	1.2800000

Early Noise-Exposed dGA=123 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	7	1.5628571	0.3667294	1.1800000	2.1100000
WAVE II	7	2.4971429	0.4721481	1.9700000	3.1500000
WAVE III	7	3.6628571	0.6954067	2.8300000	4.5400000
WAVE IV	7	4.7057143	0.4809663	4.0300000	5.3200000
TROUGH	7	6.0514286	0.2716265	5.6600000	6.3100000
AMPLITUDE	7	1.0914286	0.3852025	0.4500000	1.5700000

Early Noise-Exposed dGA=130 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	5.9000000	.	5.9000000	5.9000000
TROUGH	1	6.8400000	.	6.8400000	6.8400000
AMPLITUDE	1	0.4900000	.	0.4900000	0.4900000

Early Noise-Exposed dGA=130 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.2833333	0.2569695	2.1300000	2.5800000
WAVE II	5	3.2560000	0.3219161	2.9400000	3.6900000
WAVE III	5	4.4780000	0.3467276	4.1200000	4.9500000
WAVE IV	5	5.2860000	0.4629579	4.8300000	5.8000000
TROUGH	5	6.1640000	0.4050062	5.7900000	6.7800000
AMPLITUDE	5	0.9920000	0.5418210	0.4100000	1.7200000

Early Noise-Exposed dGA=130 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	6	1.8133333	0.1830483	1.6000000	2.0200000
WAVE II	6	2.7333333	0.2675568	2.4400000	3.0600000
WAVE III	6	3.9683333	0.2780947	3.6300000	4.2200000
WAVE IV	6	4.7400000	0.3660055	4.3100000	5.1600000
TROUGH	6	5.6850000	0.3514399	5.2600000	6.1400000
AMPLITUDE	6	1.1416667	0.6802475	0.4700000	2.1000000

Early Noise-Exposed dGA=131 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	5.7400000	.	5.7400000	5.7400000
TROUGH	1	6.8000000	.	6.8000000	6.8000000
AMPLITUDE	1	0.4600000	.	0.4600000	0.4600000

Early Noise-Exposed dGA=131 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.1833333	0.0251661	2.1600000	2.2100000
WAVE II	4	3.2150000	0.2787472	3.0000000	3.6000000
WAVE III	5	4.4580000	0.3164964	4.1400000	4.8900000
WAVE IV	5	5.2220000	0.4210938	4.8200000	5.6900000
TROUGH	5	6.1900000	0.4814561	5.7600000	6.8100000
AMPLITUDE	5	1.0400000	0.5543465	0.5700000	1.8800000

Early Noise-Exposed dGA=131 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	5	1.7760000	0.1862257	1.5800000	2.0100000
WAVE II	5	2.7180000	0.2040098	2.5400000	2.9900000
WAVE III	5	3.9300000	0.2600000	3.6700000	4.2800000
WAVE IV	5	4.7200000	0.4116430	4.3300000	5.2200000
TROUGH	5	5.5840000	0.4367265	5.1300000	6.0700000
AMPLITUDE	5	1.4040000	0.5646503	0.5800000	2.0400000

Early Noise-Exposed dGA=136 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	3	5.8000000	0.2645751	5.5000000	6.0000000
TROUGH	3	6.8000000	0.3704052	6.4200000	7.1600000
AMPLITUDE	3	0.5033333	0.2138535	0.3700000	0.7500000

Early Noise-Exposed dGA=136 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.2700000	0.1670329	2.0900000	2.4200000
WAVE II	4	3.3975000	0.2085466	3.2400000	3.6800000
WAVE III	5	4.4520000	0.2364741	4.1100000	4.7700000
WAVE IV	5	5.2800000	0.3708773	4.7300000	5.7200000
TROUGH	5	6.0560000	0.4321227	5.5000000	6.6500000
AMPLITUDE	5	0.7100000	0.3552464	0.4500000	1.3200000

Early Noise-Exposed dGA=136 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	6	1.7200000	0.2631349	1.2800000	2.0500000
WAVE II	6	2.6366667	0.3860397	1.8700000	2.8800000
WAVE III	6	3.7433333	0.5580920	2.7000000	4.1700000
WAVE IV	6	4.5200000	0.5384051	3.6800000	5.1100000
TROUGH	6	5.2733333	0.6141227	4.1800000	5.7500000
AMPLITUDE	6	1.4000000	1.0127981	0.6000000	3.2000000

Late Noise-Exposed dGA=130 Level=20

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	5.8200000	.	5.8200000	5.8200000
TROUGH	1	6.8800000	.	6.8800000	6.8800000
AMPLITUDE	1	0.2100000	.	0.2100000	0.2100000

Late Noise-Exposed dGA=130 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.8166667	0.2557994	2.5500000	3.0600000
WAVE II	3	3.7233333	0.4994330	3.4300000	4.3000000
WAVE III	3	4.6900000	0.2193171	4.4500000	4.8800000
WAVE IV	6	5.8216667	0.5882318	5.2200000	6.5800000
TROUGH	6	6.8400000	0.5997333	5.9800000	7.6600000
AMPLITUDE	6	0.4816667	0.2296447	0.2400000	0.7900000

Late Noise-Exposed dGA=130 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	9	2.2511111	0.2845367	1.8200000	2.5700000
WAVE II	9	3.0733333	0.2543619	2.7000000	3.3600000
WAVE III	11	4.1336364	0.3731025	3.7700000	4.7200000
WAVE IV	11	4.9372727	0.2840807	4.4400000	5.3600000
TROUGH	11	6.0800000	0.2338803	5.7000000	6.4000000
AMPLITUDE	11	1.1154545	0.3057569	0.6800000	1.5800000

Late Noise-Exposed dGA=130 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	7	1.8400000	0.3109662	1.4200000	2.4100000
WAVE II	7	2.7457143	0.3233603	2.3900000	3.3700000
WAVE III	6	3.8050000	0.4151024	3.3800000	4.4700000
WAVE IV	7	4.4771429	0.3967247	4.0400000	5.1800000
TROUGH	7	5.7414286	0.5068014	5.3400000	6.7600000
AMPLITUDE	7	1.3885714	0.7155517	0.6800000	2.2900000

Late Noise-Exposed dGA=131 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.8000000	0.5211526	2.4600000	3.4000000
WAVE II	3	3.9000000	0.3469870	3.6800000	4.3000000
WAVE III	3	5.0600000	0.4853864	4.6600000	5.6000000
WAVE IV	5	6.2300000	0.6675328	5.3800000	6.9000000
TROUGH	5	7.0200000	0.5904659	6.3300000	7.9200000
AMPLITUDE	5	0.5580000	0.2616677	0.2400000	0.8800000

Late Noise-Exposed dGA=131 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	10	2.4390000	0.3700285	1.8700000	2.9200000
WAVE II	11	3.4354545	0.2903916	3.0000000	3.8500000
WAVE III	11	4.3890909	0.3724635	4.0200000	5.1000000
WAVE IV	11	5.1454545	0.3213523	4.6300000	5.5800000
TROUGH	11	6.2663636	0.2765600	5.9100000	6.6900000
AMPLITUDE	11	1.0100000	0.3775977	0.4600000	1.4600000

Late Noise-Exposed dGA=131 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	7	1.8871429	0.3328020	1.4100000	2.3600000
WAVE II	7	2.9100000	0.2503331	2.6300000	3.3700000
WAVE III	7	3.9214286	0.3908294	3.4600000	4.5600000
WAVE IV	7	4.6771429	0.4270330	4.2600000	5.2900000
TROUGH	7	5.8085714	0.3322363	5.3000000	6.2400000
AMPLITUDE	7	1.2071429	0.5621007	0.4600000	1.9300000

Late Noise-Exposed dGA=136 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	3.2000000	.	3.2000000	3.2000000
WAVE II	1	4.3200000	.	4.3200000	4.3200000
WAVE III	1	5.2600000	.	5.2600000	5.2600000
WAVE IV	2	5.5800000	0.0848528	5.5200000	5.6400000
TROUGH	2	6.6650000	0.1343503	6.5700000	6.7600000
AMPLITUDE	2	0.5700000	0.4666905	0.2400000	0.9000000

Late Noise-Exposed dGA=136 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.2766667	0.2581343	1.9800000	2.4500000
WAVE II	3	3.1500000	0.2042058	2.9200000	3.3100000
WAVE III	3	4.3066667	0.2138535	4.0600000	4.4400000
WAVE IV	3	5.1333333	0.0305505	5.1000000	5.1600000
TROUGH	3	5.8433333	0.1040833	5.7600000	5.9600000
AMPLITUDE	3	0.7133333	0.1069268	0.6200000	0.8300000

Late Noise-Exposed dGA=136 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	2	1.6600000	0.1414214	1.5600000	1.7600000
WAVE II	2	2.6000000	0.1414214	2.5000000	2.7000000
WAVE III	2	3.7300000	0.1555635	3.6200000	3.8400000
WAVE IV	2	4.4800000	0.1697056	4.3600000	4.6000000
TROUGH	2	5.2200000	0.0565685	5.1800000	5.2600000
AMPLITUDE	2	0.9950000	0.2192031	0.8400000	1.1500000

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Nonexposed dGA=114 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	6.1400000	.	6.1400000	6.1400000
TROUGH	1	7.2100000	.	7.2100000	7.2100000
AMPLITUDE	1	0.2800000	.	0.2800000	0.2800000

Nonexposed dGA=114 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	1	3.2300000	.	3.2300000	3.2300000
WAVE IV	3	5.7500000	0.3200000	5.4300000	6.0700000
TROUGH	3	7.0000000	0.4350862	6.5700000	7.4400000
AMPLITUDE	3	0.4300000	0.1307670	0.3400000	0.5800000

Nonexposed dGA=116 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	5.8200000	.	5.8200000	5.8200000
TROUGH	1	8.0900000	.	8.0900000	8.0900000
AMPLITUDE	1	0.3100000	.	0.3100000	0.3100000

Nonexposed dGA=116 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	1	3.6900000	.	3.6900000	3.6900000
WAVE IV	4	5.3400000	0.5906494	4.5400000	5.8200000
TROUGH	4	6.8225000	0.4255487	6.2800000	7.3100000
AMPLITUDE	4	0.4600000	0.0752773	0.3700000	0.5500000

```
                 Nonexposed dGA=123 Level=40

Variable    N        Mean         Std Dev       Minimum       Maximum
----------------------------------------------------------------------
WAVE I      0           .               .             .             .
WAVE II     0           .               .             .             .
WAVE III    1   4.9300000               .     4.9300000     4.9300000
WAVE IV     2   6.9600000       0.5515433     6.5700000     7.3500000
TROUGH      2   8.1150000       0.6858936     7.6300000     8.6000000
AMPLITUDE   2   0.3750000       0.0212132     0.3600000     0.3900000
----------------------------------------------------------------------


                 Nonexposed dGA=123 Level=60

Variable    N        Mean        Std Dev       Minimum       Maximum
----------------------------------------------------------------------
WAVE I      0           .               .             .             .
WAVE II     1   4.4700000               .     4.4700000     4.4700000
WAVE III    2   4.7900000       1.1030866     4.0100000     5.5700000
WAVE IV     6   6.0500000       0.3372833     5.7300000     6.6700000
TROUGH      6   7.1766667       0.3544949     6.6400000     7.6000000
AMPLITUDE   6   0.5066667       0.1377921     0.2600000     0.6300000
----------------------------------------------------------------------


                 Nonexposed dGA=123 Level=80

Variable    N        Mean         Std Dev       Minimum       Maximum
----------------------------------------------------------------------
WAVE I      0           .               .             .             .
WAVE II     2   3.7750000       0.0777817     3.7200000     3.8300000
WAVE III    4   4.4100000       0.8911042     3.6200000     5.2500000
WAVE IV     7   5.3828571       0.4119350     4.7200000     5.9600000
TROUGH      7   6.6600000       0.3596294     6.1700000     7.2100000
AMPLITUDE   7   0.7485714       0.2747986     0.3800000     1.0200000
----------------------------------------------------------------------
```

Nonexposed dGA=130 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	2	6.6200000	0.4808326	6.2800000	6.9600000
TROUGH	2	7.5600000	0.2545584	7.3800000	7.7400000
AMPLITUDE	2	0.3250000	0.2757716	0.1300000	0.5200000

Nonexposed dGA=130 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	3	4.2233333	0.1050397	4.1200000	4.3300000
WAVE III	3	5.1666667	0.2112660	4.9700000	5.3900000
WAVE IV	6	5.8133333	0.1884321	5.5000000	6.0400000
TROUGH	6	6.8900000	0.4033857	6.4600000	7.6000000
AMPLITUDE	6	0.5500000	0.1861182	0.3600000	0.8400000

Nonexposed dGA=130 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	4	3.5625000	0.1806239	3.3300000	3.7200000
WAVE III	6	4.3966667	0.3959630	3.6500000	4.7900000
WAVE IV	6	5.2200000	0.2964456	4.8200000	5.6800000
TROUGH	6	6.3933333	0.3919013	6.0000000	7.1400000
AMPLITUDE	6	0.7800000	0.2991989	0.4100000	1.2300000

Nonexposed dGA=131 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	5.0100000	.	5.0100000	5.0100000
WAVE III	1	6.0400000	.	6.0400000	6.0400000
WAVE IV	1	6.2400000	.	6.2400000	6.2400000
TROUGH	1	7.3400000	.	7.3400000	7.3400000
AMPLITUDE	1	0.5300000	.	0.5300000	0.5300000

Nonexposed dGA=131 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	4.2200000	.	4.2200000	4.2200000
WAVE III	3	5.3600000	0.1385641	5.2800000	5.5200000
WAVE IV	3	5.8400000	0.2165641	5.6400000	6.0700000
TROUGH	3	6.6400000	0.0953939	6.5300000	6.7000000
AMPLITUDE	3	0.4333333	0.2145538	0.2900000	0.6800000

Nonexposed dGA=131 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	3	3.4533333	0.1939931	3.2300000	3.5800000
WAVE III	3	4.5633333	0.2600641	4.3000000	4.8200000
WAVE IV	3	5.1733333	0.3775359	4.7600000	5.5000000
TROUGH	3	6.1700000	0.3195309	5.9200000	6.5300000
AMPLITUDE	3	0.7200000	0.4703190	0.4000000	1.2600000

```
                 Nonexposed dGA=136 Level=40

Variable     N       Mean        Std Dev      Minimum      Maximum
-------------------------------------------------------------------
WAVE I       0          .            .            .            .
WAVE II      0          .            .            .            .
WAVE III     0          .            .            .            .
WAVE IV      1   6.3500000           .    6.3500000    6.3500000
TROUGH       1   7.4900000           .    7.4900000    7.4900000
AMPLITUDE    1   0.5600000           .    0.5600000    0.5600000
-------------------------------------------------------------------


                 Nonexposed dGA=136 Level=60

Variable     N       Mean        Std Dev      Minimum      Maximum
-------------------------------------------------------------------
WAVE I       0          .            .            .            .
WAVE II      2   4.1300000    0.4242641    3.8300000    4.4300000
WAVE III     0          .            .            .            .
WAVE IV      5   5.6820000    0.1661927    5.4100000    5.8600000
TROUGH       5   6.7260000    0.2059854    6.5200000    7.0300000
AMPLITUDE    5   0.7160000    0.4082034    0.4000000    1.2600000
-------------------------------------------------------------------


                 Nonexposed dGA=136 Level=80

Variable     N       Mean        Std Dev      Minimum      Maximum
-------------------------------------------------------------------
WAVE I       0          .            .            .            .
WAVE II      5   3.4660000    0.1094532    3.3300000    3.6200000
WAVE III     5   4.3380000    0.3900256    3.6500000    4.6100000
WAVE IV      5   5.0760000    0.1818791    4.8100000    5.2900000
TROUGH       5   6.0320000    0.1279453    5.8900000    6.2400000
AMPLITUDE    5   0.8980000    0.3471599    0.5900000    1.3800000
-------------------------------------------------------------------
```

Early Noise-Exposed dGA=113 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	7.2100000	.	7.2100000	7.2100000
TROUGH	1	8.6600000	.	8.6600000	8.6600000
AMPLITUDE	1	0.2200000	.	0.2200000	0.2200000

Early Noise-Exposed dGA=113 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	3.7300000	.	3.7300000	3.7300000
WAVE III	1	5.5800000	.	5.5800000	5.5800000
WAVE IV	1	6.6000000	.	6.6000000	6.6000000
TROUGH	1	8.0900000	.	8.0900000	8.0900000
AMPLITUDE	1	0.3500000	.	0.3500000	0.3500000

Early Noise-Exposed dGA=114 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	7.1700000	.	7.1700000	7.1700000
TROUGH	1	8.4800000	.	8.4800000	8.4800000
AMPLITUDE	1	0.2900000	.	0.2900000	0.2900000

Early Noise-Exposed dGA=114 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	3.7300000	.	3.7300000	3.7300000
WAVE III	2	4.4500000	1.4283557	3.4400000	5.4600000
WAVE IV	3	5.5266667	1.2606480	4.0800000	6.3900000
TROUGH	3	6.8633333	1.4257045	5.2200000	7.7700000
AMPLITUDE	3	0.3666667	0.0680686	0.2900000	0.4200000

Early Noise-Exposed dGA=116 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	3.3000000	.	3.3000000	3.3000000
WAVE III	0	.	.	.	.
WAVE IV	3	5.4466667	0.5441813	4.8200000	5.8000000
TROUGH	3	6.8400000	0.0173205	6.8200000	6.8500000
AMPLITUDE	3	0.5433333	0.1514376	0.3700000	0.6500000

Early Noise-Exposed dGA=123 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	3	6.1233333	0.3134220	5.8600000	6.4700000
TROUGH	3	7.1566667	0.3611556	6.7400000	7.3800000
AMPLITUDE	3	0.3400000	0.1044031	0.2700000	0.4600000

Early Noise-Exposed dGA=123 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	3.4700000	.	3.4700000	3.4700000
WAVE III	5	4.1580000	0.5698860	3.5300000	4.6500000
WAVE IV	6	5.4366667	0.3052649	5.1100000	6.0000000
TROUGH	6	6.6783333	0.2525404	6.4600000	7.0800000
AMPLITUDE	6	0.6400000	0.2433105	0.3800000	1.0300000

Early Noise-Exposed dGA=130 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	5	5.8120000	0.2090933	5.5100000	6.0700000
TROUGH	5	7.0540000	0.4556644	6.5500000	7.7400000
AMPLITUDE	5	0.4840000	0.1887591	0.2800000	0.7300000

Early Noise-Exposed dGA=130 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	3	3.3466667	0.3614323	3.0900000	3.7600000
WAVE III	2	2010.34	2836.43	4.6800000	4016.00
WAVE IV	5	5.2600000	0.3228777	4.8300000	5.6100000
TROUGH	5	6.2820000	0.4142704	5.8200000	6.7800000
AMPLITUDE	5	0.6980000	0.4317059	0.2500000	1.3200000

Early Noise-Exposed dGA=131 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	3	5.6600000	0.1682260	5.4700000	5.7900000
TROUGH	3	6.8166667	0.3523256	6.5300000	7.2100000
AMPLITUDE	3	0.6366667	0.3415162	0.3400000	1.0100000

Early Noise-Exposed dGA=131 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	1	2.8400000	.	2.8400000	2.8400000
WAVE III	2	4.4350000	0.4454773	4.1200000	4.7500000
WAVE IV	3	4.9833333	0.3208323	4.6500000	5.2900000
TROUGH	3	5.8433333	0.5550976	5.2500000	6.3500000
AMPLITUDE	3	0.9633333	0.5589574	0.4900000	1.5800000

Early Noise-Exposed dGA=136 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	2	6.4400000	0.6222540	6.0000000	6.8800000
TROUGH	2	7.4350000	0.7283200	6.9200000	7.9500000
AMPLITUDE	2	0.3500000	0.1555635	0.2400000	0.4600000

Early Noise-Exposed dGA=136 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	1	5.1500000	.	5.1500000	5.1500000
WAVE IV	4	5.7800000	0.3513782	5.3200000	6.1400000
TROUGH	4	6.6700000	0.2880972	6.3100000	6.9900000
AMPLITUDE	4	0.4350000	0.3630886	0.1900000	0.9700000

Early Noise-Exposed dGA=136 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	3	3.4633333	0.2345918	3.2600000	3.7200000
WAVE III	4	4.1500000	0.6734488	3.2700000	4.8800000
WAVE IV	5	5.1420000	0.4197261	4.5400000	5.6300000
TROUGH	5	6.2880000	0.4680491	5.5400000	6.8200000
AMPLITUDE	5	0.7060000	0.2166333	0.5100000	1.0700000

Late Noise-Exposed dGA=130 Level=20

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	2	7.9150000	1.1525841	7.1000000	8.7300000
TROUGH	2	8.3900000	0.8343860	7.8000000	8.9800000
AMPLITUDE	2	0.1650000	0.0777817	0.1100000	0.2200000

Late Noise-Exposed dGA=130 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	6	6.5700000	0.5921486	5.5200000	7.2400000
TROUGH	6	7.5416667	0.4203054	6.8500000	8.1300000
AMPLITUDE	6	0.3433333	0.1564182	0.1200000	0.5000000

Late Noise-Exposed dGA=130 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	4	2.8550000	0.3504759	2.5600000	3.3000000
WAVE II	6	3.9283333	0.5643905	3.4100000	4.7900000
WAVE III	6	4.8316667	0.5937143	4.2200000	5.8000000
WAVE IV	11	5.6190909	0.3948786	4.8800000	6.0000000
TROUGH	11	6.7445455	0.4250027	6.0200000	7.5100000
AMPLITUDE	11	0.6218182	0.2230613	0.3800000	1.0700000

Late Noise-Exposed dGA=130 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	1.8800000	.	1.8800000	1.8800000
WAVE II	3	3.1966667	0.3865661	2.9300000	3.6400000
WAVE III	5	4.2220000	0.6916430	3.2900000	4.9900000
WAVE IV	5	4.9380000	0.4110596	4.3700000	5.4100000
TROUGH	5	6.0980000	0.2620496	5.8600000	6.4100000
AMPLITUDE	5	0.9440000	0.3636344	0.5900000	1.5200000

Late Noise-Exposed dGA=131 Level=20

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	1	7.4900000	.	7.4900000	7.4900000
TROUGH	1	8.0400000	.	8.0400000	8.0400000
AMPLITUDE	1	0.2200000	.	0.2200000	0.2200000

Late Noise-Exposed dGA=131 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	3.6600000	.	3.6600000	3.6600000
WAVE II	1	4.5100000	.	4.5100000	4.5100000
WAVE III	2	5.8400000	0.0707107	5.7900000	5.8900000
WAVE IV	5	6.4260000	0.4026537	5.7500000	6.7400000
TROUGH	5	7.4360000	0.4875244	6.8400000	8.2000000
AMPLITUDE	5	0.3760000	0.0357771	0.3400000	0.4300000

Late Noise-Exposed dGA=131 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	4	2.9450000	0.2369951	2.7300000	3.1600000
WAVE II	5	3.7760000	0.2531403	3.5300000	4.1700000
WAVE III	7	5.0842857	0.3477479	4.6200000	5.5400000
WAVE IV	11	5.6972727	0.3677252	5.0000000	6.0700000
TROUGH	11	6.7909091	0.4984266	6.1400000	7.9200000
AMPLITUDE	11	0.6309091	0.2245642	0.2300000	1.0500000

Late Noise-Exposed dGA=131 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	3	2.5633333	0.6741167	2.1300000	3.3400000
WAVE II	4	3.3200000	0.3770942	2.9800000	3.8300000
WAVE III	4	4.4525000	0.4568278	4.0100000	5.0000000
WAVE IV	5	5.2020000	0.3798947	4.6000000	5.5400000
TROUGH	5	6.2340000	0.3482528	5.9600000	6.7100000
AMPLITUDE	5	0.9120000	0.3275973	0.5800000	1.4600000

Late Noise-Exposed dGA=136 Level=40

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	0	.	.	.	.
WAVE IV	2	6.2500000	0.5515433	5.8600000	6.6400000
TROUGH	2	7.1850000	0.2757716	6.9900000	7.3800000
AMPLITUDE	2	0.3150000	0.0494975	0.2800000	0.3500000

Late Noise-Exposed dGA=136 Level=60

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	1	3.4400000	.	3.4400000	3.4400000
WAVE II	1	4.1500000	.	4.1500000	4.1500000
WAVE III	0	.	.	.	.
WAVE IV	3	5.5266667	0.2079263	5.2900000	5.6800000
TROUGH	3	6.7600000	0.0556776	6.7100000	6.8200000
AMPLITUDE	3	0.6066667	0.1205543	0.4800000	0.7200000

Late Noise-Exposed dGA=136 Level=80

Variable	N	Mean	Std Dev	Minimum	Maximum
WAVE I	0	.	.	.	.
WAVE II	0	.	.	.	.
WAVE III	2	3.7800000	0.9758074	3.0900000	4.4700000
WAVE IV	2	4.8450000	0.3747666	4.5800000	5.1100000
TROUGH	2	6.1250000	0.0777817	6.0700000	6.1800000
AMPLITUDE	2	0.6800000	0.1555635	0.5700000	0.7900000

BIOGRAPHICAL SKETCH

Major Linda L. Pierson, U.S. Army, hails from the Adirondack Mountains, and was raised in the small mountain town of Saranac Lake, New York, before attending the State University of New York at Plattsburgh. She graduated from Plattsburgh with a B.A. degree in 1976. She obtained her M.A. degree in Audiology from New York University in 1980, and began her career as an Army audiologist that same year. As part of her career in the Army, she served as Assistant Chief of Audiology at Eisenhower Army Medical Center in Augusta, Georgia, and at Tripler Army Medical Center, Honolulu, Hawaii. Before being selected as the recipient of full scholarship for doctoral study at the University of Florida, her most recent assignment was as a hearing conservation consultant at the U. S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, Maryland. Notable achievements in the Army include the completion of Combined Arms Service and Staff School as well as Command and General Staff School. She has been awarded two Army Commendation Medals and two Meritorious Service Medals for her contributions to the hearing health of soldiers. Major Pierson’s immediate family consists of her husband James

Pierson, her daughter Carol Davis and her son Andrew Pierson.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Kenneth J. Gerhardt, Chair
Professor of Communication
Processes and Disorders

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Alice E Holmes

Alice E. Holmes
Associate Professor of
Communication Processes and
Disorders

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Scott K. Griffiths
Assistant Professor of
Communication Processes and
Disorders

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Robert M. Abrams
Professor of Obstetrics and
Gynecology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Kyle E Rarey

Kyle E. Rarey
Associate Professor of Anatomy
and Cell Biology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

William E. Bolch, Jr.
Professor of Environmental
Engineering Sciences

This dissertation was submitted to the Graduate Faculty of the Department of Communication Processes and Disorders in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1993

Dean, Graduate School

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